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Network and pathway analysis of microRNAs, transcription factors, target genes and host genes in human glioma

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Abstract. To date, there has been rapid development with regard to gene and microRNA (miR/miRNA) research in gliomas. However, the regulatory mechanisms of the associated genes and miRNAs remain unclear. In the present study, the genes, miRNAs and transcription factors (TFs) were considered as elements in the regulatory network, and focus was placed on the associations between TFs and miRNAs, miRNAs and target genes, and miRNAs and host genes. In order to show the regulatory correlation clearly, all the elements were investigated and three regulatory networks, namely the differentially-expressed, related and global networks, were constructed. Certain important pathways were highlighted, with analysis of the similarities and differences among the networks. Next, the upstream and downstream elements of differentially-expressed genes, miRNAs and predicted TFs were listed. The most notable aspect of the present study was the three levels of network, particularly the differentially-expressed network, since the differentially-expressed associations that these networks provide appear at the initial stages of cancers such as glioma. If the states of the differentially-expressed associations can be adjusted to the normal state via alterations in regulatory associations, which were also recorded in the study networks and tables, it is likely that cancer can be regulated or even avoided. In the present study, the differentially-expressed network illuminated the pathogenesis of glioma; for example, a TF can regulate one or more miRNAs, and a target gene can be targeted by one or more miRNAs. Therefore, the host genes and target genes, the host genes and TFs, and the target genes and TFs indirectly affect each other through miRNAs. The association also exists between TFs and TFs, target genes and target genes, and host genes and host genes. The present study also demonstrated self-adaption associations and circle-regulations. The related network further described the regulatory mechanism associated with glioma. These results can be utilized to adjust the states. The present study expounded the regulatory mechanisms of glioma and supplied theoretical data for further studies, in which greater attention should be focused on the highlighted genes and miRNAs.

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Abbreviations: miR/miRNA, microRNA; TFs, transcription factors; NCBI, National Center for Biotechnology Information; TFBSs, TF binding sites

Key words: glioma, transcription factor, target gene, host gene, network

Introduction

Glioma is the most commonly occurring highly malignant primary brain tumor; however, the molecular pathways resulting in glioma pathogenesis remain unclear (1). Although major advancements have been made in the management of malignant gliomas, of which glioblastomas represent the final grade of malignancy, a poor prognosis remains characteristic of the tumors (2). This poor prognosis in malignant gliomas is due to the active migration of the glioma cells, often over long distances, through the narrow extracellular spaces in the brain; this makes the cells elusive targets for effective surgical treatment (2). It has been clinically and experimentally indicated that invasive malignant glioma cells exhibit a decreased proliferation rate and a relative apoptotic resistance compared with the highly cellular tumor center; this contributes to the resistance of the tumors to conventional pro-apoptotic chemotherapy and radiotherapy (2). Apoptotic resistance is a consequence of changes at the transcriptional, post-transcriptional and genomic level of proteins, protein kinases and their transcription factor (TF) effectors (2).

TFs and microRNAs (miR/miRNAs) are the two most characterized gene regulators that have been shown to be significant in the regulation of genes. However, high throughput screening is rare for the interaction associations between TFs, miRNAs and target genes in gliomas (3). TFs promote or suppress the transcription of genes through binding to the upstream regions of the genes. TFs regulate the transcription of genes in a direct manner and occasionally cooperate with other proteins. TFs and miRNAs are therefore prominent regulators for gene expression (4). miRNAs are small non-coding RNAs that act as negative gene regulators. Alterations in miRNA expression have been indicated to be involved in the pathogenesis and development of the majority of human malignancies (5). miRNAs are

involved in numerous biological processes, including proliferation, differentiation and apoptosis. miRNAs can also target genes to regulate a number of different biological processes, thus providing experimentally validated data for associated databases. The host genes of miRNAs are the genes that the miRNAs locate to. Parallel transcription of miRNAs and their host transcripts, and two different transcriptional miRNA classes (exonic and intronic) have been identified. Intronic miRNAs and their host genes exhibit close associations (6).

The present study was centered on the associations between the elements in glioma, and the associations among these genes, miRNAs and their host genes were extracted. Three levels of networks, which were termed the differentially-expressed network, the related network and the global network were attained. The global network was constructed to represent all associations. However, this network was so complex that pathways associated with glioma could not be clearly found. Therefore, pathways concerning the differentially-expressed elements and predicted TFs were used to complete the study, which is of great significance in distinguishing the key nodes and pathways of glioma.

Materials and methods

Material collection and data processing. The miRNA and target gene datasets were collected from DIANA-Tarbase 5.0 (diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index) and miRTarBase (mirtarbase.mbc.nctu.edu.tw/). The official symbols from the National Center for Biotechnology Information (NCBI) database (ncbi.nlm.nih.gov/gene/) were used in the present study. This complete data was considered as dataset F_1.

The validated dataset of the TFs and the corresponding miRNAs regulated by these TFs was collected from TransmiR (7). This complete data was considered as dataset F_2.

The host gene miRNAs were extracted from miRbase (8) and the NCBI. Official symbols and identifications were assigned to each host gene. The complete data was considered as dataset F_3.

The differentially-expressed genes for glioma were mainly extracted from the Kyoto Encyclopedia of Genes and Genomes pathway database (www.genome.jp/kegg/pathway.html). The glioma pathway map was obtained, which shows all the validated mutated glioma genes. The relevant literature regarding the mutated genes of glioma was also manually searched. The related genes and pertinent literature were collected from the GeneCards database (http://www.genecards.org/) (9). Additionally, the popular TFs were extracted using the P-match method (10). This system combines pattern matching with weight matrix approaches, and thus provides a higher accuracy of recognition than each of the methods alone. The TFs were considered as glioma-related genes and only those that appeared in transmiR were focused upon. The 1,000-nt (5,000-nt) promoter region sequences of the targets of the differentially-expressed genes were downloaded from the University of California Santa Cruz database (11). The study used the P-match method, which combines pattern matching and weight matrix approaches, to identify transcription factor binding sites (TFBSs) in 1,000-nt promoter region sequences and the TFBSs were mapped onto the promoter region of the targets. The matrix library of P-match and the sets of known TFBSs collected in TRANSFAC also provide the possibility of searching for a large variety of different TFBSs. The vertebrate matrix and restricted high quality criterion were used for the matrix. The complete data of differentially-expressed genes and related genes was considered as dataset F_4.

Differentially-expressed miRNAs were collected from mir2Disease (12), which is a manually created database of differentially-expressed miRNAs from various human diseases. The relevant literature regarding glioma was also manually searched. The complete differentially-expressed miRNAs and related miRNAs of glioma were considered as dataset F_5.

Three levels of network construction. The regulatory associations between target genes, TFs, host genes and miRNAs were extracted from F_1, F_2 and F_3. After combining their associations, the global network was obtained. Separately, the differentially-expressed elements and related elements were extracted from F_4, and F_5, and these were mapped onto the global network. After combining these associations, the differentially-expressed network and the related network were obtained.

Results and Discussion

Differentially-expressed network of gliomas. Fig. 1 shows the significant regulatory pathways of glioma in the differentially-expressed network, which is composed of 5 TFs (PTEN, TP53, EGFR, PDGFA and TP73), target genes of miRNAs, miRNAs and their host genes. The elements were all differentially expressed, with the exception of the host genes.

In Fig. 1, a number of the specific features of TFs and miRNAs are highlighted. It can be observed that a TF can regulate one or more miRNAs, and that a target gene can be targeted by one or more miRNAs. Therefore, the host genes and target genes, the host genes and TFs, and the target genes and TFs exhibit an indirect affect on each other via the miRNAs. The association also exists between TFs and TFs, target genes and target genes, host genes and host genes. For example, PTEN regulates hsa-miR-21 and hsa-miR-25, which separately target EGFR and FBXW7. This association indicates that PTEN indirectly affects EGFR and FBXW7 by hsa-miR-21 and hsa-miR-25. In the same way, TP53 regulates hsa-miR-29c, hsa-miR-107, hsa-miR-34a and hsa-miR-125b, which all target CDK6, indicating that TP53 indirectly affects CDK6 by hsa-miR-29c, hsa-miR-107, hsa-miR-34a and hsa-miR-125b. Also, certain self-adaption associations (an miRNA is regulated by the TFs that it targets) between PTEN and hsa-miR-21, PTEN and hsa-miR-19a, TP53 and hsa-miR-125b, and EGFR and hsa-miR-21 were revealed.

Mutations were found in TP53 at 76.5% and in PTEN at 73.5%. This was considered to represent a high incidence of alterations in the cellular pathways (13). The present study highlights the significance of the development of therapeutic approaches that are viable in tumors with a wide range of genetic alterations, and provides an panel of glioma cell lines to enable this.

The features of the miRNAs and their host genes are also highlighted in Fig. 1. For example, C13orf25 includes hsa-miR-17 and hsa-miR-19a, which separately target RB1 and PTEN. Also, an miRNA may be located in one or several

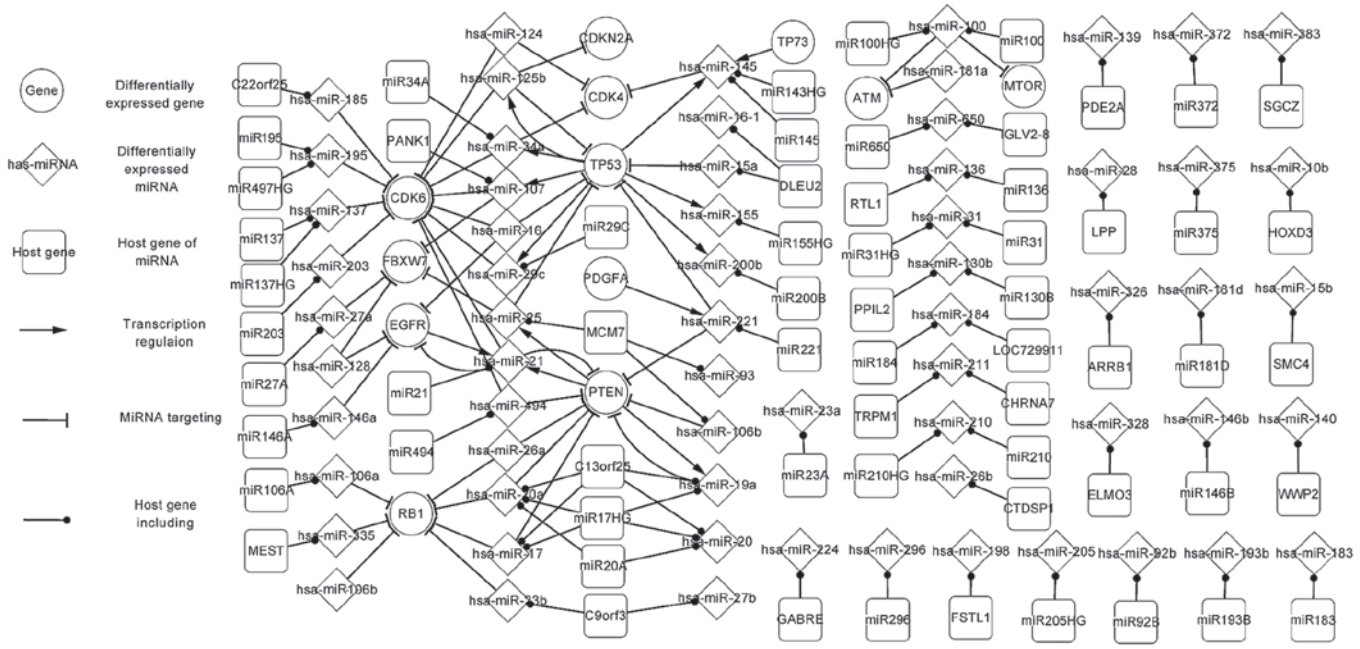

Figure 1. Differentially-expressed network of genes and miRNAs in glioma. miR/miRNA, microRNA.

genes. For example, hsa-miR-195 is located in MIR195 and MIR497HG. The differentially-expressed network partly demonstrated the regulatory mechanism of glioma.

Related network of gliomas. The related network is composed of differentially-expressed genes and miRNAs, related genes and miRNAs, and targets of miRNAs and host genes of miRNAs. So, the related network contains the differentially-expressed network. Fig. 2 shows 22 TFs, consisting of 5 differentially-expressed TFs and 17 related TFs in glioma. Apart from the differentially-expressed miRNAs, 20 related miRNAs are shown. There are numerous additional pathways. For instance, AKT1, RELA and TP53 regulate hsa-miR-155, which targets CUX1 and FADD. PDGFA regulates hsa-miR-221, which targets TP53. E2F1 and E2F3 regulate hsa-miR-15a, which targets TP53, and E2F1 and PTEN regulate hsa-miR-25, which targets TP53.

Transcriptional network of TFs and differentially-expressed miRNAs. In total, 24 differentially-expressed miRNAs, which were regulated by predicted TFs, were analyzed further. Fig. 3 presents the regulatory associations of the predicted TFs and miRNAs in glioma. These elements have an affect on their successors by targeting or regulating them. Fig. 3 demonstrates that REL, RELA and NFRB1 regulate hsa-miR-21, which targets E2F2 and E2F1. It is also possible to conclude the following: One miRNA may target a number of TFs; for example, hsa-miR-106b targets E2F1and E2F3. A TF may regulate several miRNAs; for example, E2F1 regulates hsa-miR-106a, hsa-miR-106b and hsa-miR-93. A TF indirectly affects other TFs by a number of miRNAs, and a miRNA indirectly affects other miRNAs by TFs; for example, RELA indirectly regulates CUX1 by hsa-miR-155 and hsa-miR-205 indirectly affects hsa-miR-34a by ZEB1. Certain self-adaption associations also exist between E2F1and hsa-miR-20 (hsa-miR-93, hsa-miR-19a, hsa-miR-20a, hsa-miR-17 or hsa-miR-106b). The transcription network of predicted TFs and miRNAs can therefore contribute to the study of glioma pathogenesis.

Global network of gliomas. The global network of gliomas is an experimentally validated biological network in the human body. It consists of additional TFs, targets, miRNAs and host genes of miRNA compared with the related network. The global network shows more comprehensive regulatory associations, such as those in F_1, F_2 and F_3, and includes the differentially-expressed network and the related network.

Comparison and analysis of the features of the differentially-expressed genes. All pathways of the differentially-expressed elements (genes and miRNAs) were extracted, analyzed and compared. For the differentially-expressed genes (apart from the differentially-expressed genes that did not have adjacency nodes), the interacting features of every gene were compared and analyzed by the distribution of the adjacency nodes in the three levels of network. For TFs, it was concluded that 4 types of TFs exist. For genes that do not regulate miRNAs, 3 types of genes were found.

The study first focused on the TFs. The first class of TFs, including TP53, PTEN and EGFR, has 6 types of adjacency node (3 types of successor and 3 types of predecessor). The following text focuses on PTEN and TP53.

Table I shows that 8 miRNAs target PTEN, and that PTEN regulates 3 miRNAs in the differentially-expressed network. In total, 11 and 25 miRNAs target PTEN, and PTEN regulates 3 and 10 miRNAs in the related and global networks, respectively. It was indicated that 8 miRNAs indirectly affect the expression of 3 miRNAs by PTEN in the differentially-expressed network. It was found that hsa-miR-21 targeted PTEN, and that PTEN regulated hsa-miR-21 in 3 networks, forming a self-adaption association.

Table I. Regulatory associations between miRNAs and PTEN.

Gene association	Differentially-expressed network	Related network	Global network
PTEN			
miRNAs targeting the gene	miR-106b, miR-17, miR-19a, miR-20a, miR-21, miR-221, miR-26a, miR-494	miR-106b, miR-17, miR-19a, miR-20a, miR-21, miR-214, miR-221, miR-222, miR-26a, miR-29a, miR-494, miR-216a	miR-106b, miR-141, miR-17, miR-17-5p miR-18a miR-19a, miR-19b, miR-20a, miR-21, miR-214, miR-21-5p, miR-217, miR-22, miR-221, miR-221-3p, miR-222, miR-222-3p, miR-26a, miR-29a, miR-29b-3p, miR-494, miR-519a-3p, miR-519d, miR-93-5p
miRNAs regulated by the gene	miR-19a, miR-21, miR-25	miR-19a, miR-21, miR-25	miR-19a, miR-21, miR-22, miR-25, miR-302, miR-302a, miR-302b, miR-302c, miR-302d, miR-302f

miR/miRNA, microRNA.

Table II. Regulatory associations between miRNAs and TP53.

Gene association	Differentially-expressed network	Related network	Global network
TP53			
miRNAs targeting the gene	miR-125b, miR-15a, miR-16, miR-221, miR-25, miR-25	miR-125b, miR-15a, miR-16, miR-221, miR-222, miR-16, miR-221, miR-222, miR-25, miR-30d, miR-380-5p, miR-612	miR-125a-5p, miR-125b, miR-125b-5p, miR-1285, miR-15a
miRNAs regulated by the gene	miR-107, miR-125b, miR-145, miR-155, miR-200b, miR-29c, miR-34a	miR-107, miR-125b, miR-143, miR-145, miR-155, miR-200b, miR-29a, miR-29c, miR-34a	miR-107, miR-125b, miR-125b-1, miR-125b-2, miR-143, miR-145, miR-155, miR-192, miR-194, miR-194-1, miR-194-2, miR-200a, miR-200b, miR-200c, miR-215, miR-29, miR-29a, miR-29b-1, miR-29b-2, miR-29c, miR-34, miR-34a, miR-34b, miR-34c, miR-519d

miR/miRNA, microRNA.

miR-21 has previously been shown to be associated with the proliferation and invasion of glioma. miR-21 is expressed at a higher level in glioma tissues compared with normal tissues, indicating that it could be a potential independent marker for gliomas (14), and thus a possible therapeutic target for molecular glioma therapy.

TP53 is a notable tumor suppressor and has significant features in all three levels of network. Table II shows that 5 miRNAs target TP53, and that TP53 regulates 7 miRNAs in the differentially-expressed network. A total of 6 and 12 miRNAs target TP53, and TP53 regulates 9 and 25 miRNAs in the related and global networks, respectively. It was indicated that 5 miRNAs indirectly affect the expression of 7 miRNAs by TP53 in the differentially-expressed network. It was found that hsa-miR-125b targets TP53, and that TP53 regulates hsa-miR-125b in all three levels of network, forming a self-adaption association.

The second class of TFs, including CDKN2A, has 5 types of adjacency node (2 types of successor and 3 types of predecessor). A total of 8 miRNAs target CDKN2A, which regulates has-miR-410, in the related network.

The third class of TFs, including RB1, has 4 types of adjacency node (3 types of successor and 1 type of predecessor,

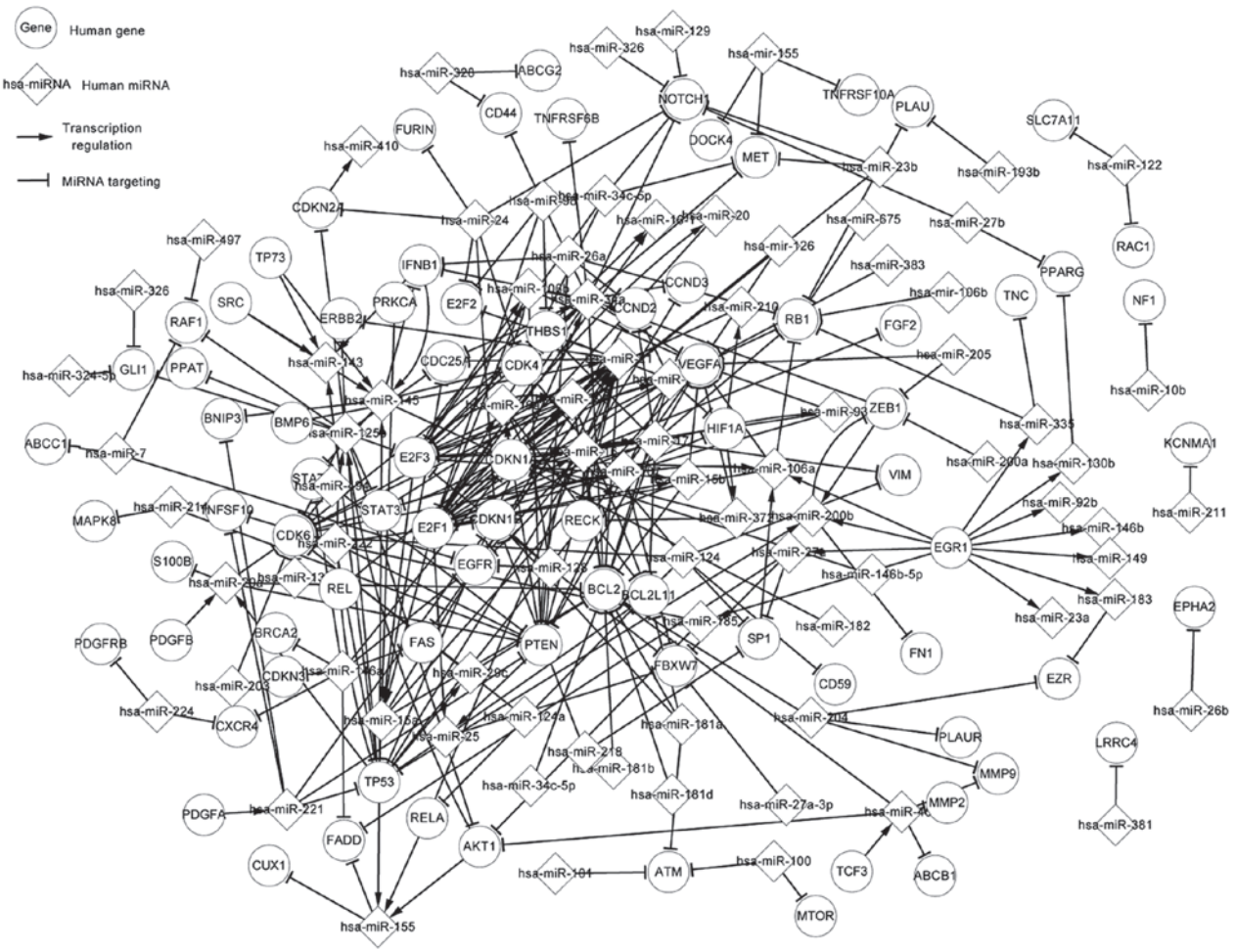

Figure 2. Related network of genes and miRNAs in glioma. miR/miRNA, microRNA.

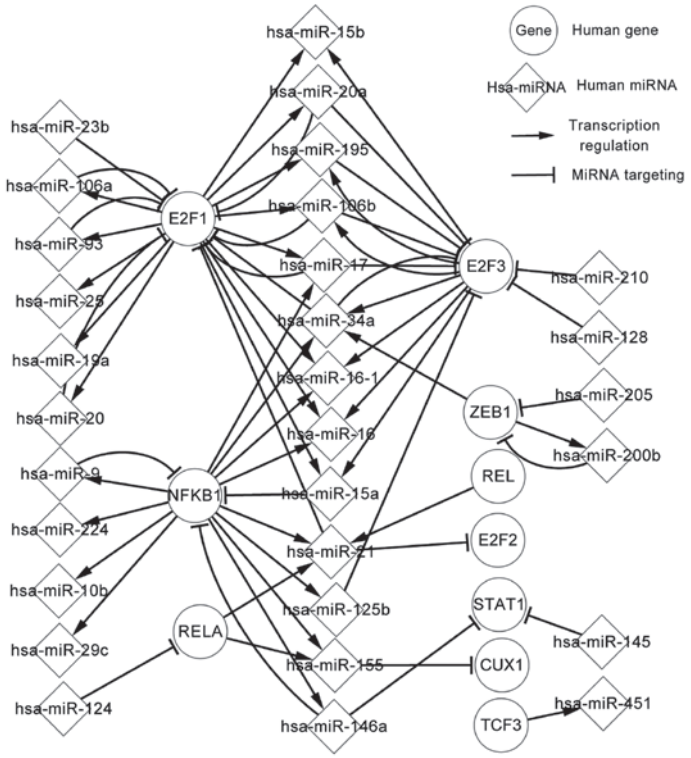

Figure 3. Transcription network of predicted transcription factors and differentially-expressed microRNAs and target genes in glioma. miR/miRNA, microRNA.

Table III. Regulatory associations between hsa-miR-21 and genes.

Regulatory association	Differentially-expressed network	Related network	Global network
hsa-miR-21			
Genes that regulate the miRNA	EGFR, PTEN	EGFR, PTEN, BMP6, REL, RELA, STAT3	EGFR, PTEN, TCF4, STAT4, STAT3, REST, RELA, REL, RASGRF1, NFKB1, NFIB, JUN, FOXO3, ETV5, ESR1, DDX5, BMPR1B, BMPR1A, BMP6
Genes targeted by the miRNA	EGFR, PTEN, CDK6	EGFR, PTEN, STAT3 RECK, FAS, ERBB2, E2F2, E2F1, CDKN1A, CDK6, CDC25A, BCL2 ACTA2	ANKRD46, APAF1, BASP1, BCL2, BMPR2, BTG2, CCR1, CDC25A, CDK2AP1, CDK6, CDKN1A, CFL2, DAXX, DERL1, E2F1, E2F2, EGFR, EIF2S1, EIF4A2, ERBB2, FAM3C, FAS, FMOD, GLCCI1, HIPK3, HNRNPK, ICAM1, IL1B, IL6R, ISCU, JAG1, JMY, LRRFIP1, MARCKS, MEF2C, MSH2, MSH6, MTAP, MYC, NCAPG, NCOA3, NFIB, NT-3, PCBP1, PDCD4, PDHA2 PELI1, PLAT, PLOD3, PPARA, PPIF, PRRG4, PTEN, PTX3, RASA1, RASGRP1, RECK, REST, RHOB, RP2, RPS7, RTN4, SERPINB5 SESN1, SGK3, SLC16A1, SOCS5, SOX5, SPATS2L, SPRY2, STAT3, TGFB1, TGFBI, TGFBR2, TGFBR3, TGIF1, TIAM1, TIMP3, TM9SF3, TNFAIP3, TOPORS, TP53BP2, TP63, TPM1, WFS1, WIBG

miR/miRNA, microRNA.

or 1 type of successor and 3 types of predecessor). A total of 15 miRNAs target RB1, which does not regulate any miRNA in the differentially-expressed network or the related network.

The fourth class of TFs, including CDKN1A and PDGFB, has 4 types of adjacency node (1 type of successor and 2 types of predecessor, or 1 type of predecessor and 2 types of successor). A total of 3 miRNAs target CDKN1A, which regulates hsa-miR-106a in the global network, but does not regulate anything in the related network.

Next, the study focused on other genes that are not TFs. The first class of gene, including ATM, CDK4, CDK6 and ATM, has 3 types of adjacency node (3 types of predecessor). CDK4 and CDK6 are only targeted by a few miRNAs in the three levels of network, but they do not regulate any miRNAs.

The second class of gene, including CDC25A and NF1, has 2 types of adjacency node in the three levels of network (2 types of predecessor). These genes are only targeted by a few miRNAs in the related and global networks, but they do not regulate any miRNAs.

The third class of gene, including BCL2L1, IDH1, SMC1A and HIST1H3B, has 1 type of adjacency node in the three levels of network (1 type of predecessor). It was indicated that these genes have the least effect compared with other differentially-expressed genes.

Comparison and analysis of the features of the differentially-expressed miRNAs. The miRNA pathways were compared and analyzed using the same method as for the regulatory pathways of the differentially-expressed genes. For 89 differentially-expressed miRNAs (12 differentially-expressed miRNAs did not have adjacency nodes), 18 differentially-expressed miRNAs (hsa-miR-9, hsa-miR-7, hsa-miR-27a, hsa-miR-21, hsa-miR-20, hsa-miR-19a, hsa-miR-195, hsa-miR-17, hsa-miR-15, hsa-miR-155, hsa-miR-146a, hsa-miR-125b, hsa-miR-200b, hsa-miR-145, hsa-miR-106b, hsa-miR-93, hsa-miR-20a and hsa-miR-34a) and their corresponding genes form self-adaption associations. That is to say that each miRNA targets TFs and is regulated by these TFs. We concluded 6 classes of miRNA, which have six, five, four, three, two or one adjacent node of miRNA, respectively. These separately included 9, 12, 14, 17, 13 and 12 miRNAs, respectively.

Table IV. Regulatory associations between miRNAs and E2F1.

Gene association	Differentially-expressed network	Related network	Global network
E2F1			
miRNAs targeting the gene	miR-106a, miR-17, miR-20, miR-20a, miR-21, miR-23b, miR-34a, miR-93	miR-106a, mir-126, miR-17, miR-20, miR-20a, miR-21, miR-23b, miR-34a, miR-93, miR-98	let-7a, miR-106a, miR-106a-5p, miR-106b, miR-126, miR-149*, miR-17, miR-17-5p, miR-20, miR-203a, miR-20a, miR-21, miR-223, miR-23b, miR-330-3p, miR-331-3p, miR-34a, miR-93, miR-98
miRNAs regulated by the gene	miR-106a, miR-106b, miR-15a, miR-15b, miR-16, miR-16-1, miR-17, miR-195, miR-19a, miR-20, miR-20a, miR-25, miR-93	miR-106a, miR-106b, miR-15a, miR-15b, miR-16, miR-16-1, miR-17, miR-195, miR-19a, miR-20, miR-20a miR-25, miR-93	let-7a, let-7a-1, let-7a-2, let-7a-3, let-7i, miR-106, miR-106a, miR-106b, miR-15a, miR-15b, miR-16, miR-16-1, miR-16-2, miR-16-3, miR-17 miR-18a, miR-18b, miR-195, miR-19a, miR-19b, miR-19b-1, miR-19b-2, miR-20, miR-20a, miR-20b, miR-223, miR-25, miR-363, miR-449, miR-449a, miR-449b, miR-449c, miR-91, miR-92-1, miR-92-2, miR-92a, miR-92a-1, miR-92a-2, miR-93

miR/miRNA, microRNA.

The first class of miRNAs, including hsa-miR-19a, hsa-miR-125b and hsa-miR-21, has 6 types of adjacency node (3 types of predecessor and 3 types of successor). The following text focuses on hsa-miR-21.

Table III shows hsa-miR-21, predecessors of hsa-miR-21 and successors of hsa-miR-21, as well as their regulatory associations. In Table III, PTEN and EGFR regulate hsa-miR-21, and the miRNA targets 3 genes in the differentially-expressed network. A total of 6 and 19 genes regulate hsa-miR-21, and the miRNA targets 12 and 87 genes in the related and global networks, respectively. It may be concluded that PTEN and EGFR indirectly affect PTEN, EGFR and CDK6 expression by regulating hsa-miR-21 in the differentially-expressed network. Table III shows that PTEN and hsa-miR-21, and EGFR and hsa-miR-21 separately form self-adaption associations. hsa-miR-21 also indirectly affects other miRNAs through certain TFs; for example, hsa-miR-21 targets PTEN, which regulates hsa-miR-19a and hsa-miR-25.

The second class of miRNAs, including hsa-miR-20a and hsa-miR-200b, has 5 types of adjacency node (3 types of successor and 2 types of predecessor, or 2 types of successor and 3 types of predecessor). No gene regulates hsa-miR-20a, but hsa-miR-20a targets PTEN and RB1 in the differentially-expressed network. E2F1 regulates hsa-miR-20a, and hsa-miR-20a targets CCND1, E2F1, RB1, PTEN, HIF1A, BCL2, CDKN1A, E2F3, THBS1 and VEGFA in the related network. TP53 regulates hsa-miR-200b, but hsa-miR-34a does not target any gene in the differentially-expressed network, A total of 3 genes regulate hsa-miR-200b, and hsa-miR-200b targets 2 genes in the related network. It was found that ZEB1 regulates hsa-miR-200b, and that hsa-miR-200b targets ZBE1 in the related network.

The third class of miRNAs, including hsa-miR-93 and hsa-miR-130b has 4 types of adjacency node (3 types of successor and 1 type of predecessor, 2 types of successor and 2 types of predecessors, or 1 type of successor and 3 types of predecessor). No TFs regulate hsa-miR-93, and hsa-miR-93 does not target any gene in the differentially-expressed network. The E2F1 gene regulates hsa-miR-93, and the miRNA targets 3 genes in the related network. E2F1 regulates hsa-miR-93, and the miRNA targets E2F1 in the global network.

The fourth class of miRNAs, including hsa-miR-10b, hsa-miR-16-1 and hsa-miR-128, has 3 types of adjacency node (3 types of successor, 2 types of successor and 1 type of predecessors, or 1 type of successor and 2 types of predecessor). It was found that no gene regulates hsa-miR-128 in all three levels of network. hsa-miR-128 targets EGFR and FBXW7 in the differentially-expressed network, and targets 4 genes in the related network. It was suggested that no gene indirectly affects the expression of other genes by regulating hsa-miR-128 in the three levels of network. If an miRNA does not have predecessors, such as hsa-miR-128, it is hypothesized that no gene regulates the miRNA in all three levels of network.

The fifth class of miRNAs, including hsa-miR-181b, hsa-miR-140 and hsa-miR-146b, has 2 types of adjacency node (2 types of successors, 1 type of successor and 1 type of predecessor, or 2 types of predecessor). No gene regulates hsa-miR-181b in all three levels of network. hsa-miR-181b targets 2 genes in the related network.

The sixth class of miRNAs, including hsa-miR-136, has 1 type of adjacency node (1 type of successor or 1 type of predecessor). No gene regulates hsa-miR-136 in the three levels of network, but the miRNA targets RIL1 in the global network. SOX9 regulates hsa-miR-140 in the global network, and hsa-miR-140 targets no genes in the three levels of network.

Comparison and analysis of the features of popular TFs. Using the same method as aforementioned, each popular TF was compared and analyzed in the three levels of network. A total of 3 TFs (E2F1, E2F3 and NFKB1) and the corresponding miRNAs were observed to form self-adaption associations.

The first class of TFs, including E2F3, E2F1, RELA, NFKB1, RUNX1, STAT3, YY1 and ZEB1, has 6 types of adjacency node (3 types of successor and 3 types of predecessor). The following text focuses on E2F1.

Table IV shows E2F1, predecessors of E2F1 and successors of E2F1, as well as their regulatory associations. In total, 8 differentially-expressed miRNAs target E2F1, and E2F1 regulates 13 differentially-expressed miRNAs. A total of 10 and 19 miRNAs target E2F1, and E2F1 regulates 13 and 31 miRNAs in the related and global networks, respectively. It was found that 5 miRNAs (hsa-miR-106a, hsa-miR-17, hsa-miR-20, hsa-miR-20a and hsa-miR-93) and E2F1 separately form self-adaption associations. E2F1 is not differentially expressed in glioma, but hsa-miR-17 and hsa-miR-93 are differentially expressed; from this it can be inferred that the two indirectly lead to E2F1 causing the abnormal expression of other miRNAs. The pathways for E2F1 and differentially-expressed miRNAs indicated further important differentially-expressed miRNAs in glioma. These miRNAs are not only differentially expressed, but also are adjacent nodes of E2F1, which is frequently involved in the transcription of cancer.

The second class of TFs, including CUX1 and TCF3, has 3 types of adjacency node (3 types of successor or 3 types of predecessor). No miRNAs target TCF3, and TCF3 regulates hsa-miR-451 in all three levels of network.

The third class of TFs, including CREB1 and NFKB1, has 2 types of adjacency node (1 type of successor and 1 type of predecessor). A total of 4 miRNAs target CREB1, and CREB1 regulates 3 miRNAs in the global network.

The fourth class of TFs, including TFAP4 and NR2F2, has 1 type of adjacency node (1 type of predecessor). hsa-miR-373 targets TFAP4 in the global network. If a TF does not havea successor, such as TFAP4, it is hypothesized that this TF does not regulate other miRNAs.

Overall, in the present study, three levels of regulatory network (the differentially-expressed, related and global networks) were collected and constructed, from which all currently experimentally validated genes and miRNAs associated with glioma were analyzed. The similarities and differences between all the differentially-expressed elements were compared in the three levels of network to distinguish the key nodes and pathways that contribute to our understanding of the carcinogenicity mechanism and therapy of glioma. Important pathways and a topological network on the development of glioma were found. Certain pathways showed unique features; for example, a TF can regulate one or more miRNAs, and a target gene can be targeted by one or more miRNAs. Therefore, the host genes and target genes, the host genes and TFs, and the target genes and TFs exhibit an indirect affect on each other via the miRNAs. The association also exists between TFs and TFs, target genes and target genes, and host genes and host genes. For example, PTEN regulates hsa-miR-21 and hsa-miR-25, which separately target EGFR and FBXW7. This association indicates that PTEN indirectly affects EGFR and FBXW7 by hsa-miR-21 and hsa-miR-25. In the same way, TP53 regulates hsa-miR-29c, hsa-miR-107, hsa-miR-34a and hsa-miR-125b, which all target CDK6, indicating that TP53 indirectly affects CDK6 by hsa-miR-29c, hsa-miR-107 and hsa-miR-34a. Also, certain self-adaption associations exist in networks between PTEN and hsa-miR-21; hsa-miR-21 targeted PTEN and PTEN regulated hsa-miR-21 in three networks. This type of association also exists between PTEN and hsa-miR-19a, TP53 and hsa-miR-125b, EGFR and hsa-miR-21, E2F1 and hsa-miR-20, E2F1 and hsa-miR-93, E2F1 and hsa-miR-19a, E2F1 and hsa-miR-20a, E2F1 and hsa-miR-17 and E2F1 and hsa-miR-106b. The results and the comprehensive data supplied in the present study will enable biologists to conduct further studies. In the future, we will consider the interaction between proteins and regulatory patterns, including upregulation and downregulation, to construct a more extensive network of glioma.

The present study highlights a number of important pathways of differentially-expressed genes, differentially expressed miRNAs and predicted TFs in glioma. The results revealed certain important pathways which have not only been observed in glioma, but also in other cancers, such as retinoblastoma. In the present study, numerous pathways were identified: For example, hsa-miR-149 targets at E2F3 and E2F3 regulates hsa-let-7i. These pathways exhibit a key biological function in retinoblastoma. Therefore, the function of the network and pathway of miRNAs, transcription factors, target genes and host genes in retinoblastoma are similar to those observed in gliomas.

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