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Construction and analysis of three networks of genes and microRNAs in adenocarcinoma

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Abstract. Adenocarcinoma is one of the most serious diseases that threaten human health. Numerous studies have investigated adenocarcinoma and have obtained a considerable amount of data regarding genes and microRNA (miRNA) in adenocarcinoma. However, studies have only focused on one or a small number of genes and miRNAs, and the data is stored in a scattered form, making it challenging to summarize and assess the associations between the genes and miRNAs. In the present study, three networks of genes and miRNAs in adenocarcinoma were focused on. This enabled the construction of networks of elements involved in adenocarcinoma and the analysis of these networks, rather than only discussing one gene. Transcription factors (TFs), miRNAs, and target and host genes of miRNAs in adenocarcinoma, and the regulatory associations between these elements were identified in the present study. These elements and associations were then used to construct three networks, which consisted of the differentially-expressed, associated and global networks. The similarities and differences between the three networks were compared and analyzed. In total, 3 notable TFs, consisting of TP53, phosphatase and tensin homolog and SMAD4, were identified in adenocarcinoma. These TFs were able to regulate the differentially-expressed genes and the majority of the differentially-expressed miRNAs. Certain important regulatory associations were also found in adenocarcinoma, in addition to self-regulating associations between TFs and miRNAs. The upstream and downstream elements of the differentially-expressed genes and miRNAs were recorded, which revealed the regulatory associations between genes and miRNAs. The present study clearly revealed components of the pathogenesis of adenocarcinoma and the regulatory associations between the elements in adenocarcinoma. The present study may aid the investigation of gene therapy in adenocarcinoma and provides a theoretical basis for studies of gene therapy methods as a treatment for adenocarcinoma.

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

Key words: three networks, transcription factors, microRNA, target genes, host genes, adenocarcinoma

Introduction

Adenocarcinoma is a malignant tumor of the glands of epithelial tissues that grows invasively and is not easy to resect (1). The rate of lymph node metastasis is relatively high in adenocarcinoma and may reach 36%. Relapse occurs easily, which leads to a poor prognosis (2). Previous studies have demonstrated that the differentially-expressed genes and miRNAs of adenocarcinoma may affect the development, transfer and treatment of cancer (3-7). In addition, there is a complex regulatory network between the TFs, miRNAs, and target and host genes in adenocarcinoma (3). Through these studies, a novel method of studying cancer may be produced. The present study aimed to investigate the elements and regulatory associations between the elements in adenocarcinoma to increase knowledge with regards to the pathogenesis, development and gene therapy of adenocarcinoma.

TFs are a type of protein that promotes or suppresses the transcription of genes by binding to the upstream regions of genes (8). TFs may regulate the transcription of genes individually or in combination with other proteins (8).

MicroRNAs (miRNAs) are short non-coding RNA sequences that demonstrate regulatory functions (8). At present, the biological function of certain miRNAs has been confirmed, which has revealed the notable regulatory role played by miRNA in the growth, differentiation and apoptosis of cells and the developmental process of disease (4). A decrease in miRNA levels is often observed in human cancers, indicating that miRNA may possess an intrinsic function of tumor suppression (9). In addition, miRNAs participate in the adjustment of cancer development by adjusting the target genes of the miRNA, which has been assessed in previous studies (4,10-12). O'Donnell *et al* revealed that the mir-17-92 gene may be associated with tumors, and also identified that mir-17-92 regulates MYC by regulating E2F1 (4).

Host genes are the genes that code for miRNAs. A considerable number of miRNAs have been identified in the introns of host genes, and these miRNAs were termed intronic miRNAs (5). The intronic miRNAs are transcribed in parallel with the host genes (5,6). Intronic miRNAs and the host genes usually act as potential partners to achieve biological function and affect the alteration of pathways (7). These previous studies have indicated that miRNA and the miRNA host genes may affect the development of cancer.

The presence of regulatory associations between TFs, miRNAs, and the target and host genes of miRNA in adenocarcinoma has been determined from the aforementioned understanding. These elements may affect the development of cancer, and a considerable number of studies have investigated these regulatory associations (3-7). From these previous studies, it has been revealed that miRNAs regulate TFs and TFs regulate miRNAs (13). It has also been demonstrated that TFs and miRNA may regulate genes (8) and miRNA may regulate target genes (10). In addition, the data of numerous studies have been combined to form various databases, including TransmiR (13), computational predicted methods (14), experimentally validated databases (15,16), miRBase (17), the KEGG pathway database (18), the miR2Disease database (19) and the GeneCards database (20). In these databases, a large amount of data may be found, which was the basis for the present study.

In the present study, TFs, miRNAs, and the host and target genes of miRNAs in adenocarcinoma were collected and analyzed. The aim of the present study was to identify the networks surrounding various elements in adenocarcinoma and to analyze these networks. Data were manually collected for adenocarcinoma, consisting of the differentially-expressed genes and miRNA, associated genes and miRNA, and the host and target genes in adenocarcinoma. The regulatory associations between the elements in adenocarcinoma were also recorded. This data formed the basis of follow-up assessments. Subsequent to the collection of various data, this data was used to construct three networks, which consisted of the differentially-expressed, associated and global networks. However, the global network was so complex that no useful data was obtained. The differentially-expressed and associated networks were considered to be more notable compared with the global network in the present study. In these networks, the key elements and pathways in adenocarcinoma were identified. Finally, the similarities and differences of the three level networks were compared and analyzed. Key elements and pathways in adenocarcinoma were then identified.

Materials and methods

Material collection and data processing

Collection of data. Initially, 3 tables, which consisted of the target genes, TFs and host genes of human miRNAs, were identified and summarized. The experimentally validated dataset obtained from Tarbase 5.0 and miRTarBase, TransmiR (13), miRBase (17) and National Center for Biotechnology Information (NCBI) was found. In order to increase the convenience of the use of the collected data, the official marks to symbolize miRNAs and genes were used. These official marks were obtained from the NCBI database. Following the collection of these data, three tables were developed, which were important components of the present study. The genes and miRNAs were then identified, as they were required to construct the three networks in the present study.

Differentially-expressed genes. The differentially-expressed genes in adenocarcinoma were obtained from the NCBI snp database (21), KEGG pathway database (18) and relevant literature.

Differentially-expressed miRNAs. The Harbin Institute of Technology miR2Disease database (19) is a manually created database of differentially-expressed miRNAs in various human diseases. This database was used in the present study to identify differentially-expressed miRNAs in adenocarcinoma. In addition, differentially-expressed miRNAs and associated miRNAs in adenocarcinoma were identified using the relevant literature (20,22-29). Following the collection of these data, differentially-expressed miRNAs from miR2Disease and the relevant literature were summarized as differentially-expressed miRNAs in adenocarcinoma. The differentially-expressed and associated miRNAs in adenocarcinoma were summarized from the relevant literature, and were classified as the associated miRNAs in adenocarcinoma.

Associated genes. The associated gene network in adenocarcinoma consisted of 4 components. Firstly, certain differentially-expressed genes in adenocarcinoma were identified using the NCBI snp database, KEGG pathway database and relevant literature (30-34). These genes formed a component of the associated gene network in adenocarcinoma. Secondly, genes were identified in adenocarcinoma using the GeneCards database (35). The genes with a relevance score >0.8, according to the GeneCards database, were extracted as a component of the associated gene network for adenocarcinoma. Thirdly, 1,000-nt promoter region sequences of the target genes of differentially-expressed genes were obtained from the University of California, Santa Cruz database (36). The P-match method, which combines pattern matching and weight matrix approaches, was then used to identify transcription factor binding sites (TFBSs) in 1,000-nt promoter region sequences and to map TFBSs onto the promoter region of target genes. This method identified certain associated genes that corresponded with miRNAs through target genes. These associated genes were a component of the associated gene network in adenocarcinoma. Finally, the last component of the associated gene network in adenocarcinoma was identified using the relevant literature (37-47). The four aforementioned components were then summarized as the associated gene network in adenocarcinoma.

Construction of the three networks. Following the collection of various data regarding adenocarcinoma, the data were used to construct three level networks, which consisted of the differentially-expressed, associated and global networks. Firstly, the global network was constructed according to the TFs, miRNAs, target genes and host genes in adenocarcinoma and the regulatory associations between these elements. Secondly, the differentially-expressed miRNAs in adenocarcinoma and the global network were used to identify the regulatory associations between the differentially-expressed miRNAs and the host genes. Differentially-expressed genes and miRNAs and the global network were used to find the regulatory associations between the differentially-expressed genes and

differentially-expressed miRNAs. The differentially-expressed network consisted of these regulatory associations. Finally, in the same way, the associated genes and miRNAs were used to construct the associated network of adenocarcinoma.

Results

Differentially-expressed network of adenocarcinoma. Fig. 1 presents the differentially-expressed network of adenocarcinoma and reports the regulatory associations between the differentially-expressed genes and miRNAs, and target and host genes in adenocarcinoma. In this network, all the elements were differentially-expressed, with the exception of the host genes of miRNAs, and all the pathways between these elements have been experimentally validated. In total, 4 TFs, consisting of EGFR, phosphatase and tensin homolog (PTEN), SMAD4 and TP53, were identified in the differentially-expressed network of adenocarcinoma. The regulatory associations between these TFs and miRNAs are important. Therefore, these TFs and the corresponding regulatory associations with miRNAs were focused on.

In the differentially-expressed network, TP53 was found to directly regulate 9 miRNAs, consisting of hsa-miR-29b-1, hsa-miR-192, hsa-miR-194, hsa-miR-200a, hsa-miR-34a, has-miR-34b, hsa-miR-34c, hsa-miR-145 and hsa-miR-143. TP53 may also regulate an additional 4 genes, consisting of CTNNB1, MET, FHIT and KRAS, indirectly by regulating these 9 miRNAs. This reveals that genes may regulate other genes by regulating miRNA. TP53 regulates hsa-miR-143 and hsa-miR-145, which are regulated by SMAD4. SMAD4 is regulated by hsa-miR-26a. hsa-miR-26a and other miRNAs regulate the expression of PTEN. PTEN regulates 3 miRNAs, consisting of hsa-miR-21, hsa-miR-22 and hsa-miR-302b. These phenomena reveal that miRNAs may regulate genes individually or in combination with other miRNAs, and miRNAs may regulate other miRNAs by regulating genes. In addition, self-regulating associations exist between the EGFR and PTEN genes and hsa-miR-21. Therefore, PTEN and EGFR may indirectly regulate each other by regulating hsa-miR-21.

Regulatory associations between differentially-expressed genes and differentially-expressed miRNAs in adenocarcinoma are reported clearly in this network. Out of the three networks, the differentially-expressed network is the most notable network, as understanding the pathogenesis of adenocarcinoma is of considerable use. In addition, the differentially-expressed network possesses a more noteworthy significance, as it is known that the most important elements in adenocarcinoma are those in the differentially-expressed network. When the number of genes and miRNAs in the differentially-expressed network are at a normal level, individuals do not develop adenocarcinoma. If the number of genes and miRNAs in the differentially-expressed network are at an abnormal level, it is possible that adenocarcinoma may develop. Once the number of key elements in the differentially-expressed network of an adenocarcinoma patient is controlled properly, in order to return the number to a normal level, the differentially-expressed network may return to the normal state through the pathways in the network. Therefore, adenocarcinoma may be successfully treated. This forms the principle of gene therapy.

The differentially-expressed network in the present study revealed that TP53, PTEN and SMAD4 are extremely important TFs. These TFs regulate differentially-expressed genes and the majority of the differentially-expressed miRNAs in adenocarcinoma directly or indirectly. This provides a theoretical basis for studies investigating gene therapy as a treatment method for adenocarcinoma. Careful investigation of the differentially-expressed network and an understanding of the regulatory associations between the elements in adenocarcinoma may result in the successful treatment of adenocarcinoma by appropriately controlling the levels of key elements in the differentially-expressed network.

Associated network of adenocarcinoma. The method used to construct the differentially-expressed network was also used to construct the associated network of adenocarcinoma. The associated network revealed differentially-expressed genes and miRNAs, associated genes and miRNAs, and target and host genes in adenocarcinoma, in addition to the regulatory associations between these elements. The elements and pathways in the differentially-expressed network are included in the associated network.

In the associated network, there were 23 associated TFs in addition to the 4 differentially-expressed TFs, 18 associated miRNAs in addition to the 71 differentially-expressed miRNAs, and numerous additional pathways to those included in the differentially-expressed network. One example is the self-regulating associations between the MYC and E2F1 genes and hsa-miR-17 in the associated network. Therefore, MYC and E2F1 may regulate each other. Differentially-expressed genes and miRNAs play an important role in the development of adenocarcinoma, but these associated genes and associated miRNAs may also affect the pathogenesis and development of adenocarcinoma. Thus, the construction and investigation of the associated network may aid the understanding of the pathogenesis of adenocarcinoma.

Global network of adenocarcinoma. Genes, miRNAs, target genes and host genes and the regulatory associations between these elements were used to construct the global network. The global network contains all the elements and pathways that were included in the differentially-expressed or associated networks. The global network is the most complex network out of the three networks.

In order to describe the networks of adenocarcinoma more clearly, the upstream and downstream information of the important elements was extracted from this network.

Regulatory associations between differentially-expressed genes. The predecessor and successor nodes of the differentially-expressed genes in adenocarcinoma were extracted from the three networks. These extracted genes revealed that the predecessor and successor nodes surrounding differentially-expressed genes demonstrate evident ladder characteristics in the three networks. This clearly demonstrated the regulatory associations between the differentially-expressed genes in adenocarcinoma.

In total, 30 differentially-expressed genes were identified in adenocarcinoma using the aforementioned method (data not shown). Overall, 12 genes did not possess adjacent nodes.

Table I. Regulatory associations between miRNAs and PTEN.

	Network		
Association	Differentially-expressed	Associated	Global
Targets PTEN	hsa-miR-141	hsa-miR-141	hsa-miR-141
	hsa-miR-21	hsa-miR-21	hsa-miR-21
	hsa-miR-214	hsa-miR-214	hsa-miR-214
	hsa-miR-217	hsa-miR-217	hsa-miR-217
	hsa-miR-221	hsa-miR-221	hsa-miR-221
	hsa-miR-222	hsa-miR-222	hsa-miR-222
	hsa-miR-26a	hsa-miR-26a	hsa-miR-26a
		hsa-miR-17	hsa-miR-17
		hsa-miR-216a	hsa-miR-216a
		hsa-miR-29b	hsa-miR-29b
			hsa-miR-18a
			hsa-miR-19a
			hsa-miR-19b
			hsa-miR-19b-1
			hsa-miR-19b-2
			hsa-miR-20
			hsa-miR-20a
			hsa-miR-216
			hsa-miR-26a-1
			hsa-miR-26a-2
			hsa-miR-494
			hsa-miR-519a
			hsa-miR-519d
			hsa-miR-91
			hsa-miR-93
Regulated by PTEN	hsa-miR-21	hsa-miR-21	hsa-miR-21
	hsa-miR-302b	hsa-miR-302b	hsa-miR-302b
	hsa-miR-22	hsa-miR-22	hsa-miR-22
		hsa-miR-25	hsa-miR-25
			hsa-miR-19a
			hsa-miR-302
			hsa-miR-302a
			hsa-miR-302c
			hsa-miR-302d
			hsa-miR-302f

Those genes that possessed adjacent nodes were analyzed in the present study. In total, 4 differentially-expressed TFs, which are extremely important elements in adenocarcinoma, were identified. Each demonstrated 6 types of adjacent node, with 3 types of predecessor nodes and 3 types of successor nodes. Only PTEN was focused on as an example, however.

PTEN demonstrated significant features in the three networks, as reported in Table I. In the differentially-expressed network, 7 miRNAs targeted PTEN and PTEN regulated 3 miRNAs. It is hypothesized that the 3 successors of PTEN are regulated indirectly by the 7 predecessors through PTEN. In addition, if the 3 successors of PTEN regulate other genes, PTEN may regulate more genes indirectly by regulating these 3 successors. Therefore, PTEN plays an important role in the pathogenesis and development of adenocarcinoma. There are similar features in the associated and global networks. In addition, hsa-miR-21 targets PTEN and PTEN regulates hsa-miR-21 in return. Therefore, there is a self-regulating association between hsa-miR-21 and PTEN.

In addition, it is known that the adjacent miRNAs in the differentially-expressed network or associated network may affect the pathogenesis and development of adenocarcinoma. In addition to these miRNAs, there are 6 other miRNAs in the global network that are not included in the differentially-expressed or associated networks. The effect of these miRNAs on the pathogenesis and development

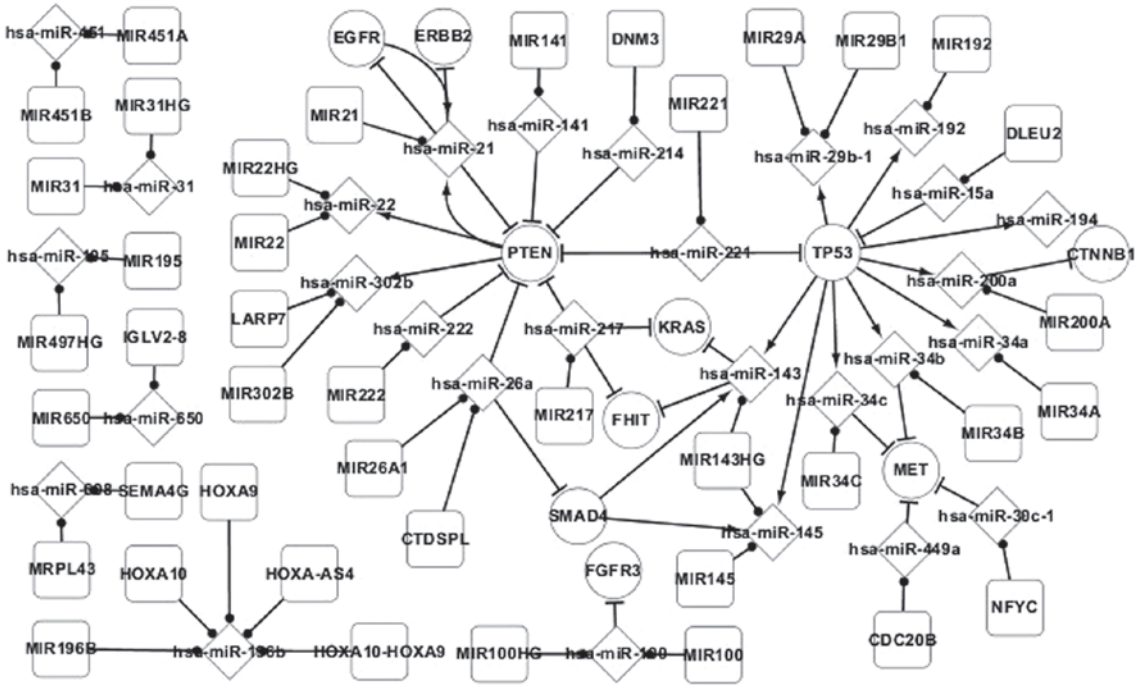

Figure 1. Differentially-expressed network of adenocarcinoma.

of adenocarcinoma remains unknown. Additional studies investigating these miRNAs are required to increase the understanding of adenocarcinoma. This may aid additional understanding of the pathogenesis of adenocarcinoma.

Regulatory associations between differentially-expressed miRNAs. The predecessor and successor nodes of the differentially-expressed miRNAs in adenocarcinoma were extracted from the three networks in order to analyze the regulatory associations between the differentially-expressed miRNAs.

In total, 71 differentially-expressed miRNAs in adenocarcinoma were identified using this method, 24 of which did not possess adjacent nodes (data not shown). Numerous other elements in adenocarcinoma may be regulated by 6 notable miRNAs with 6 types of adjacent node in a three-level network. However, only hsa-miR-143 was discussed as an example in the present study.

In the differentially-expressed network, 2 genes, consisting of SMAD4 and TP53, were found to regulate hsa-miR-143, and hsa-miR-142 was found to target the FHIT and KRAS genes. In the associated network, 6 genes were found to regulate hsa-miR-143, which, in turn, targeted 4 genes. In the global network, 12 genes regulated hsa-miR-143, with hsa-miR-143 targeting a total of 14 genes (Table II). Therefore, it is hypothesized that the SMAD4 and TP53 genes regulate the FHIT and KRAS genes indirectly, by regulating hsa-miR-143 in the differentially-expressed network. Similar phenomena were identified in the associated and global networks.

Regulatory associations between TFs. The predecessor and successor nodes of the TFs in adenocarcinoma were extracted from the three networks. This method identified 27 TFs, 15 of which did not possess adjacent nodes (data not shown). In total, 15 of the TFs possessed adjacent nodes, but only E2F3 was discussed as an example in the present study.

E2F3 was found to demonstrate significant phenomena in the three networks reported in Table III. In the differentially-expressed network, 3 miRNAs, consisting of hsa-miR-195, hsa-miR-34a and hsa-miR-34c, target the E2F3 gene. In turn, E2F3 regulates hsa-miR-195, hsa-miR-34a and hsa-miR-15a. In the associated network, 5 miRNAs target E2F3 and E2F3 regulates 3 miRNAs. In the global network, 18 miRNAs were found to target E2F3, and E2F3 was found to regulate 14 miRNAs. It is hypothesized that certain miRNAs regulate other miRNAs indirectly through E2F3. In addition, in the differentially-expressed, associated and global networks, 2 miRNAs, consisting of hsa-miR-195 and hsa-miR-34a, targeted E2F3, and E2F3 regulated these miRNAs in return. It is hypothesized that there are self-regulating associations between hsa-miR-195 and hsa-miR-34a and E2F3.

miRNA and host gene network in adenocarcinoma. The mutation of host genes is known to possibly affect the miRNAs in these host genes. Therefore, the regulatory associations between host genes and miRNAs require investigation. The host genes and the regulatory associations between the host genes and miRNAs were extracted from the differentially-expressed network to construct Fig. 2.

Regulatory associations were identified between the host genes and miRNAs in adenocarcinoma (Fig. 2). miRNA is regulated by host genes or other miRNA sequences, such as the regulation of hsa-miR-22 by PTEN, and miRNA also targets genes, such as the targeting of PTEN by hsa-miR-222. Host genes may code for several miRNAs, such as MIR143HG coding for hsa-miR-143 and hsa-miR-145, and one type of miRNA may locate to several host genes, such as hsa-miR-26a locating to MIR26A1 and CTDSPL. In addition, there are

Table II. Regulatory associations between genes and hsa-miR-143.

	Network		
Association	Differentially-expressed	Associated	Global
Regulates hsa-miR-143	SMAD4	SMAD4	SMAD4
	TP53	TP53	TP53
		SMAD3	SMAD3
		SRC	SRC
		TGFB1	TGFB1
		TP73	TP73
			BRD2
			CEBPB
			IFNB1
			IFNG
			JAG1
			KLF2
Targeted by hsa-miR-143	FHIT	FHIT	FHIT
	KRAS	KRAS	KRAS
		FSCN1	FSCN1
		HRAS	HRAS
			COL1A1
			DNMT3A
			FNDC3B
			HK2
			MACC1
			MAPK12
			MAPK7
			MT-CO2
			MYO6
			SERPINE1

self-regulating associations between genes, including EGFR and PTEN, and hsa-miR-21.

Transcriptional network of popular TFs. The regulatory associations between TFs and differentially-expressed miRNAs in adenocarcinoma are revealed in Fig. 3, which contains 4 TFs, consisting of TP53, EGFR, PTEN and SMAD4. The TFs regulate the transcription of genes individually or with other proteins. Certain associations were identified between TFs and miRNAs. It was found that miRNA may be regulated by several TFs, such as hsa-miR-143 being regulated by SMAD4 and TP53, and TFs may be targeted by several differentially-expressed miRNAs, such as hsa-miR-15a and hsa-miR-221 targeting TP53. In addition, TFs regulate other TFs indirectly by regulating differentially-expressed miRNAs. For example, PTEN regulates EGFR indirectly by regulating hsa-miR-21. miRNAs also regulate other miRNAs indirectly by regulating TFs. For example, hsa-miR-15a regulates hsa-miR-192 indirectly by regulating TP53. In addition, self-regulating associations were identified between the EGFR and PTEN genes and hsa-miR-21. Therefore, PTEN and EGFR may regulate each other. The regulatory associations between TFs and differentially-expressed miRNAs may aid the understanding of the pathogenesis of adenocarcinoma and may also aid the investigation of gene therapy methods.

Discussion

Previous studies have predicted the existence of regulatory associations between the TFs, miRNAs, and target and host genes of miRNAs in adenocarcinoma, and these elements may affect the development of cancer (3-7,9). However, the regulatory associations between the elements have not been reported at present.

In the present study, the three networks were used to collect and analyze these regulatory associations. The TFs, miRNA, and host and target genes of miRNA in adenocarcinoma were collected and the regulatory associations between these elements were determined. Three level networks of adenocarcinoma were constructed to identify key elements and pathways in adenocarcinoma. The upstream and downstream data of the notable elements was then extracted in order to describe the network of adenocarcinoma more clearly.

In total, 4 key TFs, consisting of EGFR, PTEN, SMAD4 and TP53, were identified in adenocarcinoma. TP53, PTEN and SMAD4, in particular, are extremely important in adenocarcinoma. In the differentially-expressed network, these TFs

Table III. Regulatory associations between E2F3 and miRNAs.

Association	Network		
	Differentially-expressed	Associated	Global
Targets E2F3	hsa-miR-195	hsa-miR-195	hsa-miR-195
	hsa-miR-34a	hsa-miR-34a	hsa-miR-34a
	hsa-miR-34c	hsa-miR-34c	hsa-miR-34c
		hsa-miR-17	hsa-miR-17
		hsa-miR-200b	hsa-miR-200b
			hsa-miR-106b
			hsa-miR-125b
			hsa-miR-125b-1
			hsa-miR-125b-2
			hsa-miR-128
			hsa-miR-128-1
			hsa-miR-128-2
			hsa-miR-20
			hsa-miR-203a
			hsa-miR-20a
			hsa-miR-210
			hsa-miR-34
			hsa-miR-91
Regulated by E2F3	hsa-miR-195	hsa-miR-195	hsa-miR-195
	hsa-miR-34a	hsa-miR-34a	hsa-miR-34a
	hsa-miR-15a	hsa-miR-15a	hsa-miR-15a
			hsa-let-7a
			hsa-let-7a-1
			hsa-let-7a-2
			hsa-let-7a-3
			hsa-let-7i
			hsa-miR-106b
			hsa-miR-15b
			hsa-miR-16
			hsa-miR-16-1
			hsa-miR-16-2
			hsa-miR-34

regulate differentially-expressed genes and the majority of the differentially-expressed miRNAs in adenocarcinoma directly or indirectly. This result is supported by a previous study, in which it was concluded that TFs may regulate genes (8). The differentially-expressed network revealed that TFs affect the pathogenesis and development of adenocarcinoma. In addition, 14 other differentially-expressed genes were identified. In the differentially-expressed network, these genes are targeted by miRNAs and affect the development of adenocarcinoma. The associated network contains numerous genes and miRNAs that are not included in the differentially-expressed network. These elements cannot affect adenocarcinoma as evidently as the differentially-expressed genes and miRNAs, but they continue to affect the pathogenesis and development of adenocarcinoma, and require investigation.

Prior to the present study, the data regarding adenocarcinoma was scattered across various databases, including TransmiR (13), computational predicted methods (42), experimentally validated databases (15,16), miRBase (17), the KEGG pathway database (18), the miR2Disease database (19), the GeneCards database (20) and numerous relevant studies (13,17-20,22-47). The analysis and use of this data is challenging. In the current study, a considerable amount of data was collected, which was identified and used to construct three networks. The pathogenesis of adenocarcinoma was clearly revealed and the key elements and pathways surrounding the elements in adenocarcinoma were identified through the three networks. It may be hypothesized that controlling key elements and pathways appropriately to ensure genes and miRNAs in the differentially-expressed network do not mutate may prevent healthy individuals from developing adenocarcinoma. The regulation of a small number of key elements in patients with adenocarcinoma, resulting in the return of the whole network to a normal state through the pathways between genes and miRNA, adenocarcinoma is likely

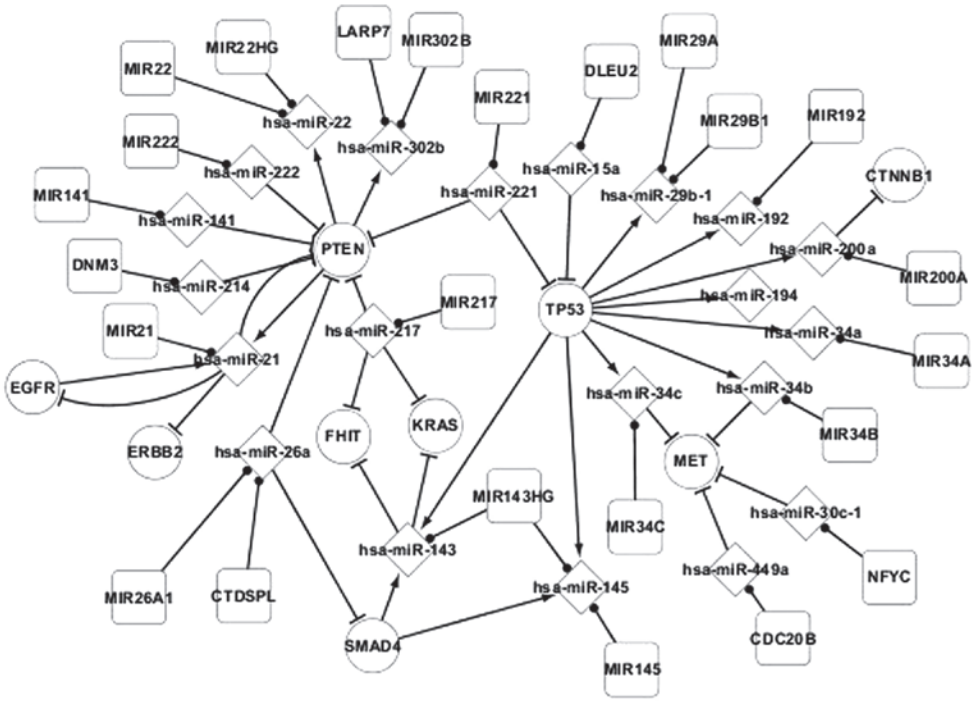

Figure 2. Regulatory associations between host genes and microRNAs in adenocarcinoma.

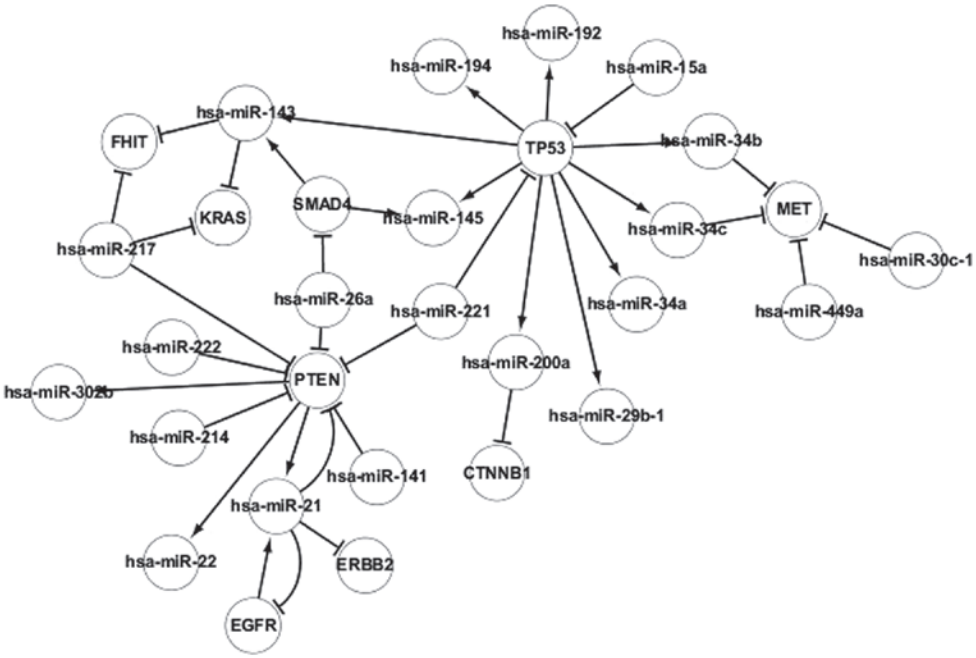

Figure 3. Transcriptional network of prominent transcriptional factors.

to be treated successfully. Therefore, the present study may aid the investigation of gene therapy for adenocarcinoma and paves the way for the successful treatment of adenocarcinoma.

In conclusion, numerous studies have identified the regulatory associations between various elements in adenocarcinoma, but failed to collect and analyze these regulatory associations. In the present study, numerous data in various databases and relevant literatures were collected to construct the three networks. Using these networks, 4 notable TFs were identified and the important associations between various elements were analyzed. The three networks clearly revealed regulatory associations between various elements in adenocarcinoma, which is important for the gene therapy of adenocarcinoma.

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