

Bioinformatics analysis of key genes and latent pathway interactions based on the anaplastic thyroid carcinoma gene expression profile

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Abstract. Anaplastic thyroid carcinoma (ATC) is an aggressive malignant disease in older adults with a high mortality rate. The present study aimed to examine several key genes and pathways, which are associated with ATC. The GSE33630 gene expression profile was downloaded from the Gene Expression Omnibus database, which included 11 ATC and 45 normal thyroid samples. The differentially expressed genes (DEGs) in ATC were identified using the Limma package in R. The Gene Ontology functions and Kyoto Encyclopedia of Genes and Genomes pathways of the selected DEGs were enriched using the Database for Annotation, Visualization and Integrated Discovery. A protein-protein interaction (PPI) network of the DEGs was constructed to select significant modules. Furthermore, a latent pathway interactive network was constructed to select the significant pathways associated with ATC. A total of 665 DEGs in the ATC samples were screened, and four significant modules were selected from the PPI network. The DEGs in the four modules were enriched in several functions and pathways. In addition, 29 significant pathways associated with ATC were selected, and the Toll-like receptor (TLR) signaling pathway, extracellular matrix (ECM)-receptor interaction and cytokine-cytokine interaction pathway were identified as important pathways. FBJ murine osteosarcoma viral oncogene homolog (FOS), chemokine C-X-C motif ligand 10 (CXCL10), collagen type V α 1 (COL5A1) and chemokine (C-C motif) ligand 28 (CCL28) were the key DEGs involved in these significant pathways. The data obtained in the present study revealed that the TLR signaling pathway, ECM-receptor interaction and cytokine-cytokine receptor interaction pathway, and the FOS, CXCL10, COL5A1, COL11A1 and CCL28 genes

have different roles in the progression of ATC, and these may be used as therapeutic targets for ATC.

Introduction

Thyroid cancer is one of the most common types of malignancy of the endocrine system (1). Anaplastic thyroid carcinoma (ATC), one of the four types of thyroid carcinoma, is an uncommon but aggressive malignancy in older adults, with a morbidity rate of 1.0-7.5%, however, it accounts for 14-39% of thyroid carcinoma-associated mortality and the mean survival duration of ATC is usually <6 months from diagnosis (2). Although certain novel treatment methods, including surgery, genetic therapy and differentiation therapy, provide possibilities for the treatment of ATC (3), there is no effective systemic therapy for ATC. Therefore, it is important to examine the molecular mechanisms of ATC and identify several treatment methods for patients with a diagnosis of ATC.

The etiology of ATC remains to be fully elucidated. Previous studies have demonstrated that ionizing radiation, abnormal iodine intake, genetic factors and autoimmune disease are the primary factors contributing to the progression of ATC (4). Passaro *et al* (5) reported that ionizing radiation enhances the cell death of oncolytic adenovirus dl922-947 in ATC. Increased iodine intake contributes to a lower incidence of ATC (6), and it has been reported that forkhead box o3a enhances the proliferation of ATC cells via regulating the transcription factor, cyclin A1 (7). In addition, several pathways involved in the progression of ATC have been identified, including the Notch1 signaling pathway, phosphoinositide 3-kinase/Akt signaling pathway and the epidermal growth factor receptor/extracellular signal-regulated kinase pathway (8-10).

With developments in biology, several studies have focussed on to the mechanism of ATC. Consequently, several biomarkers have been identified for its treatment. For example, paired-box gene 8 may be a useful biomarker for ATC (11). In a study by Kim *et al* (12) L1 cell adhesion molecule was found to be overexpressed in patients with ATC and it may be an important therapeutic target for ATC treatment. In addition, the mutation of TP53 and expression of SRY-box 2 are associated with the progression from papillary thyroid carcinoma (PTC) to ATC (13). However, the mechanism underlying ATC remains to be fully elucidated.

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Key words: anaplastic thyroid carcinoma, latent pathway analysis, differentially expressed gene, significant pathway, protein-protein interaction network

Microarray analysis is an effective approach to monitor global alterations of gene expression and identify the genes important to ATC. Several studies have been performed using the mRNA expression profile, GSE33630, of thyroid carcinoma. For example, Hébrant *et al* (14) found that ATC and PTC overlapped. He *et al* (15) analyzed 13 genes and one pathway associated with ATC. In addition, Xu *et al* found nine genes and one miRNA, which were identified as candidates for the progression of thyroid cancer (16). However, the latent pathway interactions of ATC remain to be fully elucidated. Thus, using the same samples, the present study aimed to analyze the differentially expressed genes (DEGs) and significant pathways of ATC. Comprehensive bioinformatics analysis was used to enrich the significant functions and pathways of the DEGs to provide detailed insight into the biological mechanisms of ATC. This approach was used to predict the hub genes most likely associated with ATC. The present study aimed to provide a basis for further investigation of the mechanism of ATC.

Materials and methods

Data resources and data preprocessing. The GSE33630 gene expression profile data was downloaded from the Gene Expression Omnibus database (<https://www.ncbi.nlm.nih.gov/geo/>) from the National Center for Biotechnology Information based on the platform of the GPL570 Affymetrix Human Genome U133 Plus 2.0 array. The present study included a total of 105 samples, which comprised 11 ATC samples, 49 PTC samples and 45 normal thyroid (NT) samples. The 11 ATC samples and 45 NT samples were selected for analysis in the present study.

The CEL files were transformed into the expression value matrix using the Affy package in R (17), and the probe information was then transformed into the gene name using Bioconductor in R (18). If one gene had more than one probe, the mean expression value of this gene was selected.

DEG screening and enrichment analysis. The Limma package (19) in R language was used to select the DEGs in the ATC samples, compared with the NT samples. $|\log_2 \text{fold-change}| > 2$ and adjusted $P < 0.05$ were selected as the threshold values.

Gene Ontology (GO) analysis has become a commonly used approach for functional investigations of large-scale genomic or transcription data (20). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database contains information regarding how molecules or genes are networked, which provides complementary information to the majority of the existing molecular biology databases containing information on individual genes (21). The Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics resources consist of an integrated biological knowledge base and analytic tools aimed at systematically extracting biological meaning from large gene or protein lists (22). In the present study, the GO functions and pathways of the selected DEGs were enriched using DAVID in the GO (<http://www.geneontology.org>) and KEGG (<http://www.genome.ad.jp/kegg/>) databases with the adjusted P-value (q-value) of < 0.05 .

PPI interaction network construction and selection of significant modules. The PPI interactive pairs of the screened DEGs were selected using the STRING online database (<http://string-db.org/>) (23). The DEG interactive pairs with a combined score of > 0.8 were selected for the PPI network construction. Cytoscape (<http://www.cytoscape.org/>) was used to construct the PPI interaction network (24). The MCODE plugin was used to select the significant modules associated with ATC from the PPI interaction network (25). In addition, the GO terms and pathways of the DEGs in significant modules were enriched using DAVID (22) with $P < 0.05$.

Latent pathway identification analysis (LPIA). LPIA, developed by Pham *et al* (26), is a method for identifying the interactions of pathways associated with DEGs. A significant interaction represents a correlation between pathways and disease. The LPIA process was as follows: i) GO biological process (BP) terms (G) and KEGG pathways (P) of the DEGs were calculated using clusterProfiler (27) in R. ii) A bipartite network was constructed between G and P, one edge of the node was G and the other edge of node was P, with an edge representing one gene involved in G and P, the weight of the edge was determined by the relative overlap of G and P, which was calculated using Jaccard similarity coefficient and the mean expression value for the expression of each DEG. The formula to determine the weight was as follows:

$$w_{GP} = \frac{|G \cap P|}{|G \cup P|} \times \text{med}\{DE_x : x \in G \cap P\}$$

where P represents the pathway, G represents the GO BP term of each gene, DE represents the expression value of the DEG,

$\frac{|G \cap P|}{|G \cup P|}$ is the Jaccard similarity coefficient of G and P and

$|G \cup P|$ is the total DEGs associated with G and P. iii) Based on the bipartite network, the pathways connected with at least one BP term were selected to construct the pathway network. The formula used for the weight of the edge was as follows:

$$A_{ij} = \sum_{k=1}^G w_{G_k P_i} \times w_{G_k P_j};$$

iv) The random walk method (28) was used to calculate the interaction of each pathway pair, and the significant interactions were selected. The transfer matrix of the random walk method was as follows:

$$T_{ij} = \frac{A_{ij}}{\sum_j A_{ij}}.$$

N_p represents the total pathways in the network, T_{ij} represents the probability of one pathway between P_i and P_j .

The process was repeated using the bootstrap method (29) steps 1-4, following which the significant P-value of the interaction was obtained.

Results

Data preprocessing and screening of DEGs. A total of 19,944 expression probes were obtained from the GSE33630

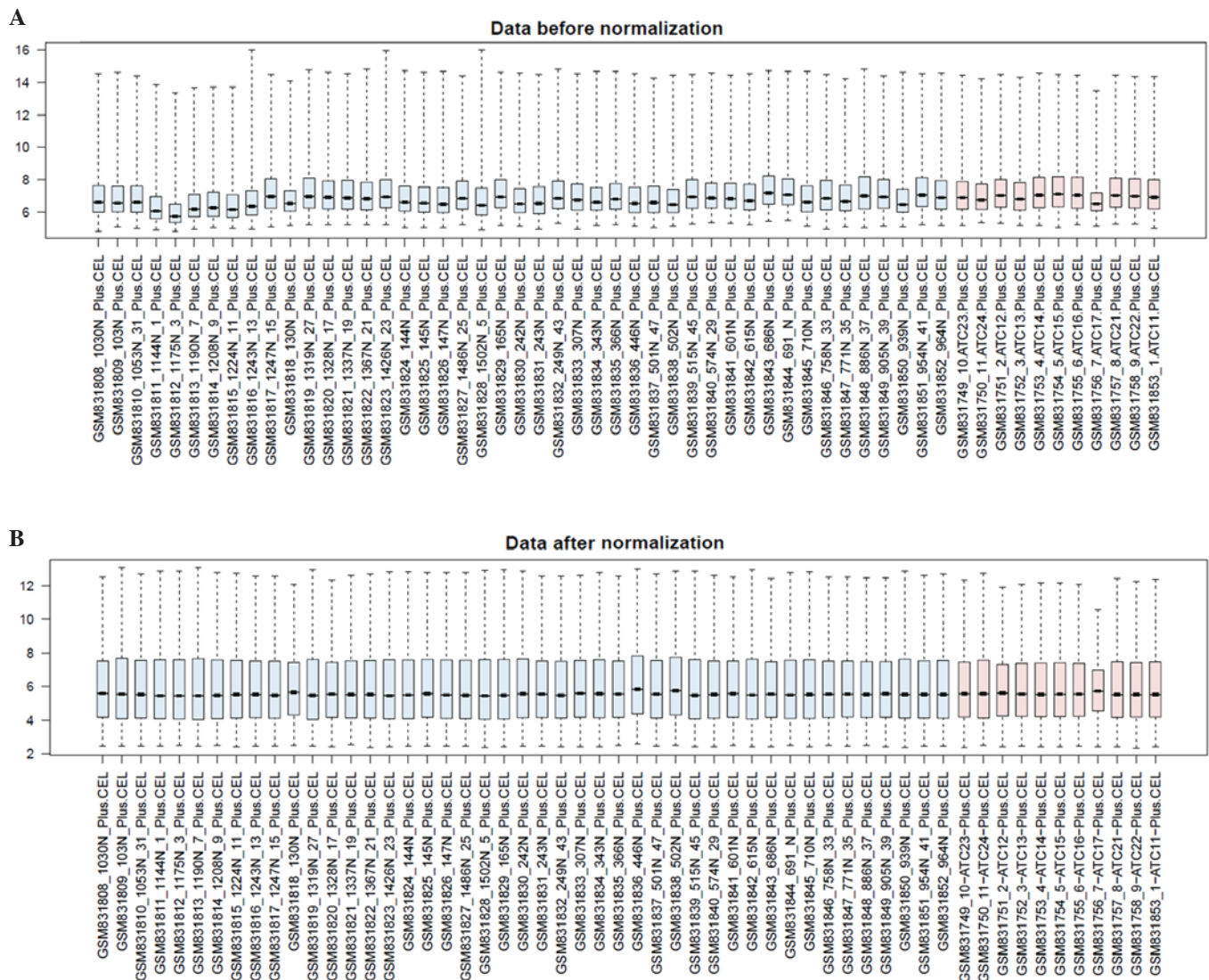


Figure 1. Box figure of gene expression data prior to and following normalization. (A) Data prior to normalization; (B) Data following normalization. The horizontal axis represents the sample and the vertical axis represents the expression value. Black lines indicate the median values. Blue represents the control samples and red represents the anaplastic thyroid carcinoma samples. The vertical axes represent expression signal values.

gene expression profile. The preprocessed data is shown in Fig. 1A and B. The median values indicated that the data were well normalized.

In addition, 665 genes in the ATC samples, which were differentially expressed compared with the NT samples were screened, which comprised 380 upregulated and 285 down-regulated DEGs.

GO functions and pathways enrichment analysis of DEGs.

The GO terms and pathways of all the selected DEGs were enriched using clusterProfiler with $P < 0.05$ (Table I). The enriched GO terms for the upregulated DEGs in ATC included cellular processes, membrane part and protein binding, whereas single-organism cellular process, extracellular region part and microtubule binding were enriched terms for the down-regulated DEGs. In addition, the upregulated DEGs were involved in glycerophospholipid metabolism and metabolic pathways (Table IA), whereas the down-regulated DEGs were involved in cytokine-cytokine receptor interaction and the complement and coagulation cascades pathway (Table IB).

PPI interaction network and significant modules. A PPI interaction network of the DEGs was constructed using STRING. A total of four significant modules with the top five node degree were selected (Fig. 2A-D). Functional enrichment analysis for the four modules found that no GO term or pathway was enriched by genes in module D. The enriched GO terms and pathways of the DEGs in these significant modules are shown in Table II.

Interaction analysis between pathways. A total of 406 pathway pairs associated with the DEGs were selected, which were involved in 29 pathways (Fig. 3; Table III). Edges with a high weight indicated a higher level of interaction between two pathways. The interactive pathway pairs with the top five weights are shown in Table IV. hsa04620 interacted with several pathways with high weights, including hsa05323, hsa05142 and hsa05146, indicating hsa04620 was an important pathway. hsa04060 was also identified as an important pathway, which interacted with several other pathways. As shown in Table IV, FBJ murine osteosarcoma viral oncogene homolog (FOS) was the DEG present

Table I. Top five enriched GO terms and pathways of the DEGs.

ID	Term	Description	Count	P-value
A, Top five GO terms and pathways of the upregulated DEGs in ACT				
BP	GO:0008150	Biological process	264	4.79E-21
BP	GO:0044707	Single-multicellular organism process	126	1.84E-06
BP	GO:0044699	Single-organism process	203	1.84E-06
BP	GO:0009987	Cellular process	230	1.84E-06
BP	GO:0032501	Multicellular organismal process	127	5.99E-06
CC	GO:0005575	Cellular component	284	2.26E-06
CC	GO:0016020	Membrane	162	9.97E-05
CC	GO:0044425	Membrane part	131	0.00042334
CC	GO:0044459	Plasma membrane part	54	0.00042334
CC	GO:0005615	Extracellular space	30	0.00042334
MF	GO:0003674	Molecular function	247	9.38E-17
MF	GO:0005515	Protein binding	139	7.67E-05
MF	GO:0005488	Binding	195	0.000128045
MF	GO:0016491	Oxidoreductase activity	22	0.004818428
MF	GO:0008509	Anion transmembrane transporter activity	11	0.00600053
KEGG	hsa00564	Glycerophospholipid metabolism	6	0.005474129
KEGG	hsa01100	Metabolic pathways	30	0.009706045

B, Top five GO terms and pathways of the downregulated DEGs in ACT

BP	GO:0044763	Single-organism cellular process	213	1.08E-26
BP	GO:0044699	Single-organism process	222	4.03E-26
BP	GO:0000278	Mitotic cell cycle	55	7.10E-22
BP	GO:0006950	Response to stress	108	2.27E-21
BP	GO:0008150	Biological process	242	5.54E-20
CC	GO:0044421	Extracellular region part	69	8.67E-25
CC	GO:0005576	Extracellular region	88	2.08E-20
CC	GO:0031012	Extracellular matrix	37	2.79E-18
CC	GO:0005615	Extracellular space	50	1.69E-17
CC	GO:0005578	Proteinaceous extracellular matrix	33	5.96E-17
MF	GO:0003674	Molecular function	234	4.95E-16
MF	GO:0005515	Protein binding	161	5.84E-16
MF	GO:0005488	Binding	203	4.91E-12
MF	GO:0008017	Microtubule binding	16	1.28E-09
MF	GO:0015631	Tubulin binding	17	1.45E-08
KEGG	hsa04512	Extracellular matrix-receptor interaction	14	7.14E-10
KEGG	hsa05150	<i>Staphylococcus aureus</i> infection	8	8.10E-06
KEGG	hsa04510	Focal adhesion	14	1.24E-05
KEGG	hsa04060	Cytokine-cytokine receptor interaction	16	1.44E-05
KEGG	hsa04610	Complement and coagulation cascades	8	1.56E-05

ACT, anaplastic thyroid carcinoma; DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function; CC, cellular component.

in hsa04620, the Toll-like receptor (TLR) signaling pathway and COL5A1 was the DEG present in hsa04512 (ECM-receptor interaction). Certain cytokines, including CXCL12, CXCL3, interleukin (IL)8, CXCL10 and CCL28, were the genes involved in hsa04060 (cytokine-cytokine receptor interaction pathway).

Discussion

ATC is an aggressive malignancy of older adults with a low morbidity rate, but a high mortality rate (2). Investigating the mechanism of ATC is beneficial to the treatment and diagnosis

Table II. Top five enriched GO terms and pathways of differentially expressed genes in the significant modules.

ID	Term	Description	Count	P-value
A, Top five enriched GO terms and pathways of DEGs in module A				
BP	GO:0007049	Cell cycle	57	5.95E-49
BP	GO:0000278	Mitotic cell cycle	49	5.95E-49
BP	GO:0022402	Cell cycle process	52	2.58E-47
BP	GO:0051301	Cell division	36	7.19E-37
BP	GO:0000280	Nuclear division	32	8.81E-36
CC	GO:0005819	Spindle	23	2.20E-26
CC	GO:0015630	Microtubule cytoskeleton	31	5.69E-23
CC	GO:0000793	Condensed chromosome	18	3.83E-22
CC	GO:0000775	Chromosome, centromeric region	16	8.06E-20
CC	GO:0000777	Condensed chromosome kinetochore	13	7.98E-19
MF	GO:0005515	Protein binding	56	6.33E-13
MF	GO:0008017	Microtubule binding	12	6.33E-13
MF	GO:0015631	Tubulin binding	12	1.81E-11
MF	GO:0003777	Microtubule motor activity	8	4.83E-10
MF	GO:0005524	ATP binding	23	6.79E-10
KEGG	hsa04110	Cell cycle	8	8.20114825913878e-10
B, Top five enriched GO terms and pathways of DEGs in module B				
BP	GO:0006935	Chemotaxis	11	6.44E-14
BP	GO:0042330	Taxis	11	6.44E-14
BP	GO:0006954	Inflammatory response	9	4.30E-11
BP	GO:0040011	Locomotion	11	1.21E-10
BP	GO:0009605	Response to external stimulus	11	1.54E-10
CC	GO:0005615	Extracellular space	7	9.36E-06
CC	GO:0044421	Extracellular region part	7	3.43E-05
CC	GO:0005576	Extracellular region	8	0.000169765
CC	GO:0044459	Plasma membrane part	6	0.004168711
CC	GO:0005886	Plasma membrane	8	0.012325713
MF	GO:0008009	Chemokine activity	7	3.55E-15
MF	GO:0042379	Chemokine receptor binding	7	5.71E-15
MF	GO:0001664	G-protein coupled receptor binding	7	3.54E-11
MF	GO:0005125	Cytokine activity	7	4.17E-11
MF	GO:0005126	Cytokine receptor binding	7	4.20E-11
KEGG	hsa04062	Chemokine signaling pathway	8	7.14E-10
KEGG	hsa04060	Cytokine-cytokine receptor interaction	8	8.10E-06
C, Top five enriched GO terms and pathways of DEGs in module C				
BP	GO:0030198	Extracellular matrix organization	13	1.57E-16
BP	GO:0043062	Extracellular structure organization	13	1.57E-16
BP	GO:0022617	Extracellular matrix disassembly	9	5.02E-15
BP	GO:0030574	Collagen catabolic process	8	1.12E-13
BP	GO:0044243	Multicellular organismal catabolic process	8	1.93E-13
CC	GO:0044421	Extracellular region part	18	4.69E-16
CC	GO:0031012	Extracellular matrix	13	8.60E-15
CC	GO:0005581	Collagen	9	8.60E-15
CC	GO:0005578	Proteinaceous extracellular matrix	12	4.04E-14
CC	GO:0044420	Extracellular matrix part	10	4.80E-14

Table II. Continued.

ID	Term	Description	Count	P-value
MF	GO:0005515	Protein binding	17	0.002325685
KEGG	hsa05142	Chagas disease (American trypanosomiasis)	7	1.8408112078959e-08

DEGs, differentially expressed genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; MF, molecular function; CC, cellular component.

Table III. 29 enriched significant pathways in the latent pathway identification analysis network.

Path ID	Description
hsa04512	Extracellular matrix-receptor interaction
hsa04974	Protein digestion and absorption
hsa04060	Cytokine-cytokine receptor interaction
hsa05150	<i>Staphylococcus aureus</i> infection
hsa04610	Complement and coagulation cascades
hsa05146	Amoebiasis
hsa05323	Rheumatoid arthritis
hsa04380	Osteoclast differentiation
hsa04062	Chemokine signaling pathway
hsa04620	Toll-like receptor signaling pathway
hsa04145	Phagosome
hsa04640	Hematopoietic cell lineage
hsa04510	Focal adhesion
hsa04670	Leukocyte transendothelial migration
hsa05144	Malaria
hsa04350	Transforming growth factor- β signaling pathway
hsa05142	Chagas disease (American trypanosomiasis)
hsa04110	Cell cycle
hsa04971	Gastric acid secretion
hsa05412	Arrhythmogenic right ventricular cardiomyopathy
hsa04514	Cell adhesion molecules
hsa00564	Glycerophospholipid metabolism
hsa05414	Dilated cardiomyopathy
hsa00240	Pyrimidine metabolism
hsa05200	Pathways in cancer
hsa01100	Metabolic pathways
hsa04020	Calcium signaling pathway
hsa04010	Mitogen-activated protein kinase signaling pathway
hsa04080	Neuroactive ligand-receptor interaction

of ATC. In the present study, the key genes and significant pathways associated with ATC were analyzed using ATC and NT samples from the GSE33630 gene expression profile. Several key genes, including CXCL10, FOS, COL5A1 and IL8, and the TLR signaling pathway, ECM-receptor interaction and cytokine-cytokine receptor interaction pathway, in which

these key genes were involved, were identified using LPIA. However, due to the different selection methods and samples, the findings of the present study were different from those of previous studies, which used the same gene expression profile (GSE33630) of thyroid cancer. Hébrant *et al* (14) reported that a more marked EMT was the qualitative difference between PTC and ATC, by examining 11 ATC and 48 PTC samples in GSE33630. He *et al* (15) found that the p53 signaling pathway, which included 13 genes, was important for thyroid cancer using gene set enrichment and meta-analysis. Xu *et al* (16) selected the BCL2, MRPS31, ID4, RASAL2, DLG2, MYO1B, ZBTB5, PRKCQ, PPP6C genes and an miRNA on considering NT, ATC and PTC together. Therefore, further experimental validation is required.

Certain types of cancer are associated with inflammatory or infectious diseases, and inflammatory factors are associated with ATC. CXCL10 is a chemokine, which produces a ligand for the receptor, CXCR3, and has a paralog of CXCL9 (30). Graves' disease is an autoimmune thyroid disorder of thyroid cancer (31). The pathogenesis of autoimmune thyroiditis and Graves' disease have been associated with the secretion of CXCL10 stimulated by T helper 1 lymphocytes (32). In addition, Antonelli *et al* (33) confirmed that human ATC cells produce CXCL10 under the effect of certain cytokines. Thus, autoimmune thyroiditis may contribute to the progression of ATC via the secretion of CXCL10. By contrast, the TLR signaling pathway may act as the novel target for the therapy of inflammatory-associated disease (34), however, the role of the TLR signaling pathway in ATC remains to be fully elucidated. Mardente *et al* (35) reported that the late inflammatory cytokine, high-mobility group box 1, can signal danger to the immune system via the TLR in PTC cell growth. The serum levels of CXCL10 are high in patients with Graves' disease, and CXCL10 and CXCL9 are important in the recruitment of thyroid cells (36). The data obtained in the present study showed that the TLR signaling pathway interacted with the cytokine-cytokine pathway, and the downregulated expression of CXCL10 was involved in the two pathways. This indicated that these two pathways were important in the progression of ATC and that CXCL10 may act as a therapeutic target of ATC.

The present study also showed that the downregulation of IL-8 was important in module B and in the TLR signaling pathway, suggesting it may be important to ATC. IL-8 is a CXC chemokine, which has the antimicrobial functions, and can activate the chemotaxis of neutrophils, T lymphocytes and eosinophils (37). Several cytokines have been found to be associated with thyroid cancer. The transfection of IL-12 in the thyroid can potentially inhibit tumor

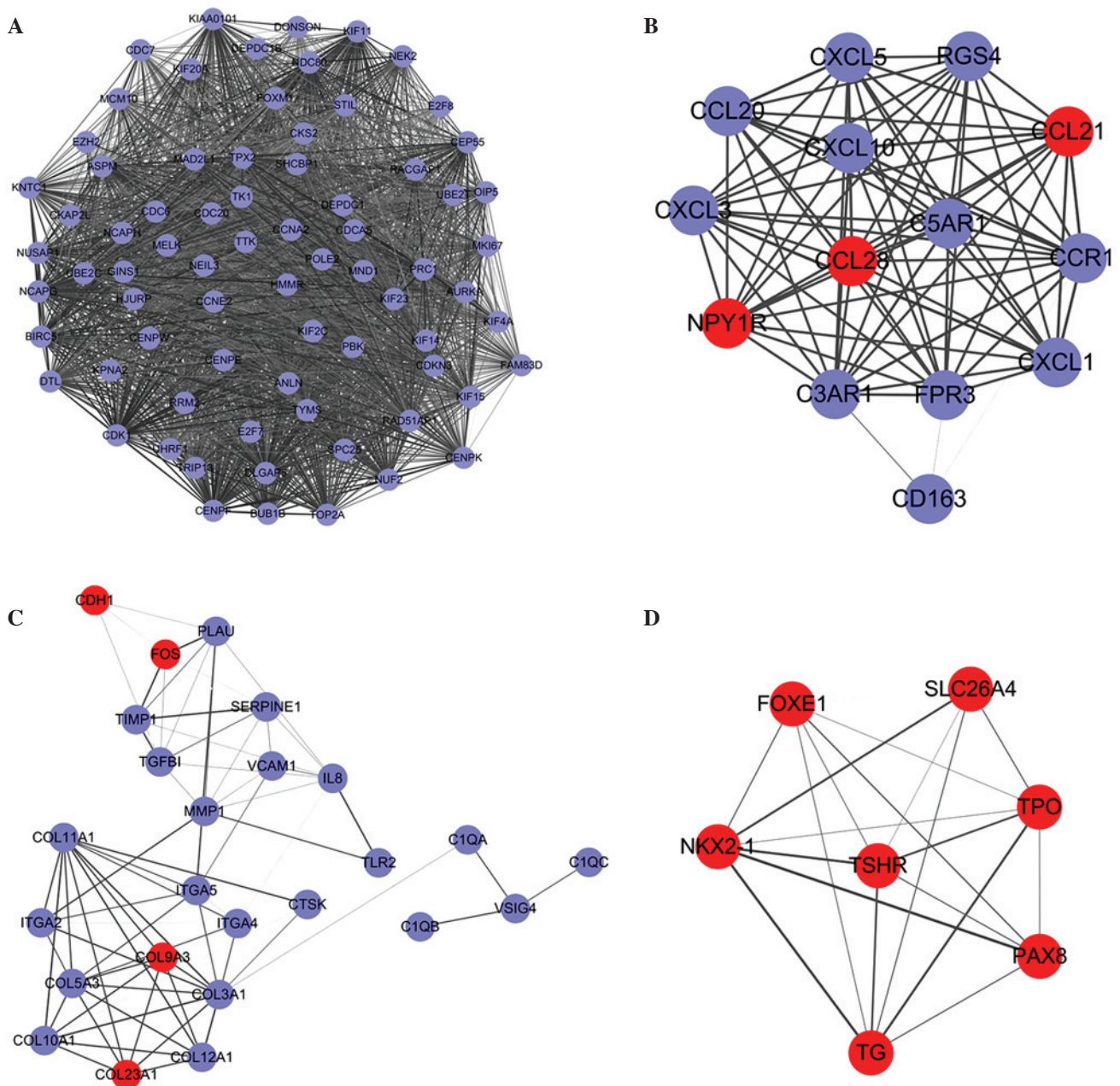


Figure 2. Modules containing the top five node degrees in the protein-protein interaction network. Red indicates upregulated DEGs, purple indicates downregulated DEGs. Modules A-D are the four significant modules. DEGs, differentially expressed genes. Lines between DEGs represent interactions between them.

growth and may be a potential tumor vaccine (38). In a study by Kobawala *et al* (39) the serum levels of IL-8 in patients with ATC were higher, compared with those in healthy individuals, and it may be a target for the diagnosis and therapeutic treatment of ATC. The findings of the present study indicated that the downregulation of IL-8 assisted in the evasion of immune surveillance, leading to ATC. Therefore, IL-8 may be a biomarker for the diagnosis of ATC.

FOS is a member of the FOS family, which acts as a regulator of cell proliferation, differentiation and apoptotic cell death (40). The role of FOS in ATC remains to be fully elucidated, however, studies have demonstrated that c-FOS can bind to the promoter, cellular FLICE-inhibitory protein, to function in prostate cancer cells (41). c-FOS signaling is regulated by protein kinase R in the promotion of cell

proliferation in hepatocellular cancer (42). The cell proliferation instability-related gene cluster is an important factor in ATC transformation (43). Thus, the FOS protein may be crucial in ATC formation. In the present study, the upregulation of FOS was present in the TLR signaling pathway, suggesting that FOS may be important in contributing to ATC formation via the TLR signaling pathway.

ECM is a protein, which is crucial in thyroid tumor invasion, metastasis and diagnosis (44). COL, a low abundance fibrillar collagen of the COL family, is a component of ECM (45). The activity of base membrane COL4 indicates the cell proliferation and metastatic abilities of thyroid cancer (46). Kusunoki *et al* (47) showed that the level of COL4 collagenase in patients with thyroid cancer was higher, compared with that in the normal thyroid, and COL4 may assist in predicting

Table IV. Interacting pairs of pathways with top 10 weights.

Pathway 1 and description	Pathway 2 and description	Weight	Co-genes
hsa05323: Rheumatoid arthritis	hsa04620: Toll-like receptor signaling pathway	0.492651941	CTSK ^a /CTSO ^a /FOS/IL8 ^a /TLR2 ^a
hsa05142: Chagas disease (American trypanosomiasis)	hsa04620: Toll-like receptor signaling pathway	0.464920784	IL8 ^a /TLR2 ^a /FOS
hsa05146: Amoebiasis	hsa04620: Toll-like receptor signaling pathway	0.37284676	IL8 ^a /TLR2 ^a /CD14 ^a
hsa04620 Toll-like receptor signaling pathway	hsa04060: Cytokine-cytokine receptor interaction	0.351558647	IL8 ^a /CXCL10 ^a
hsa04062: Chemokine signaling pathway	hsa04060: Cytokine-cytokine receptor interaction	0.33933744	CCR1 ^a /CXCL1 ^a /CXCL3 ^a /IL8 ^a /CXCL10 ^a /CCL28/CCL8 ^a /CCL13 ^a /CCL18 ^a /CCL21/CXCL5 ^a
hsa04380: Osteoclast differentiation	hsa04620: Toll-like receptor signaling pathway	0.327230722	CTSK ^a /FOS
hsa04974: Protein digestion and absorption	hsa04512: ECM-receptor interaction	0.31737287	COL5A1 ^a /COL6A3 ^a /COL11A1 ^a /COL5A3 ^a
hsa04512: ECM-receptor interaction	hsa04510: Focal adhesion	0.307854633	COL5A1 ^a /COL6A3 ^a /COL11A1 ^a /COMP ^a /TNC ^a /IBSP ^a /ITGA2 ^a /ITGA4 ^a /ITGA5 ^a /LAMB3 ^a /COL5A3 ^a /RELN/SPP1 ^a /THBS2 ^a /
hsa04145: Phagosome	hsa04620: Toll-like receptor signaling pathway	0.305699295	TLR2 ^a /CD14 ^a
hsa05150: <i>Staphylococcus aureus</i> infection	hsa04610: Complement and coagulation cascades	0.272281647	CFD/C1QA ^a /C1QB ^a /C1QC ^a /C3AR1 ^a /C5AR1 ^a

^aDownregulated differentially expressed genes. ECM, extracellular matrix.

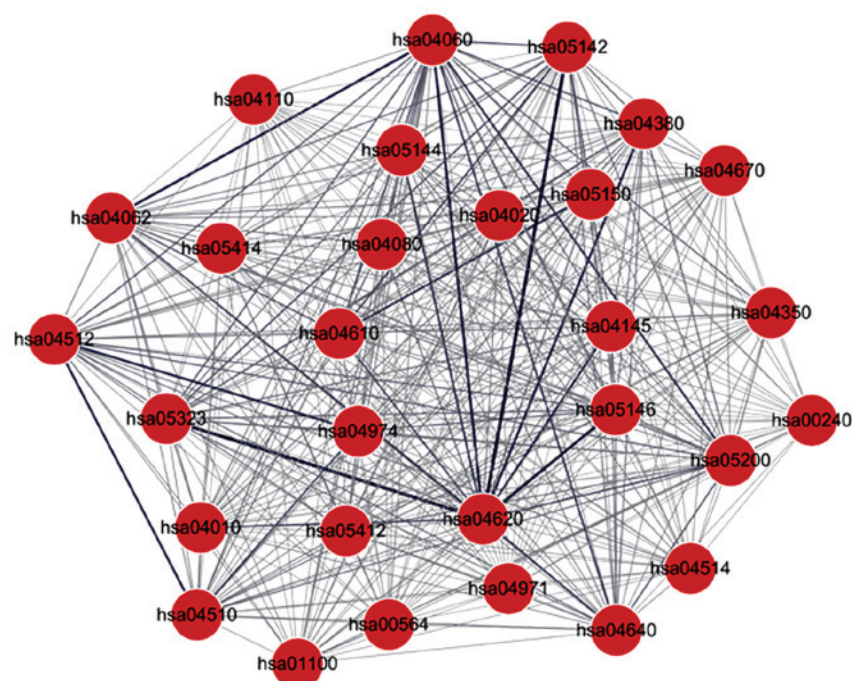


Figure 3. Interaction network of the pathways involved with the differentially expressed genes. Lines between DEGs represent interactions between them.

the invasion and metastasis of thyroid cancer. The expression of COL5A1 is suppressed by microRNA-145 in anaplastic meningiomas (48). Downregulated COL5A1 can decrease the adhesion of tumor cells via the ECM-receptor interaction pathway, enhancing tumor metastasis (49). COL11A1 and COL5A1 promote cell migration and tumor progression in mice (50). In the present study, the findings showed that COL5A1 and COL11A1 were downregulated and were present in the ECM-receptor interaction pathway, suggesting that the two genes may be important in the progression of ATC via the ECM-receptor interaction pathway.

In conclusion, the present study analyzed the key genes and significant pathways in ATC, compared with NT samples. The TLR signaling pathway, ECM-receptor interaction and cytokine-cytokine receptor interaction pathway were found to be closely associated with ATC. CXCL10 may be of benefit in ATC treatment, FOS may be involved in ATC formation, COL5A1 and COL11A1 may promote the progression of ATC, and IL8 may act as a biomarker for the diagnosis of ATC. The results of the present study provide a basis for further clinical molecular target therapy experiments for ATC.

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