

Integrated bioinformatics analysis of apolipoprotein M in thyroid cancer

jiang wei

Soochow University <https://orcid.org/0000-0002-0943-3705>

Yang Yu

Soochow University

Jun. Zhang

Soochow University

Ning Xu

Lunds Universitet

Guanghua Luo (✉ shineroar@163.com)

<https://orcid.org/0000-0001-8339-2828>

Research article

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Abstract

Background Throid cancer is one of the most common cancer worldwide and its mechanism of development remains elusive. Apolipoprotein M (ApoM) is associated with lipid metabolism, inflammation and atherosclerosis, but the prognostic value of apoM in thyroid cancer has not been well studied.

Methods In this study, transcriptional expression, survival, gene ontology and networks of apoM in patients with thyroid cancer were analyzed using integrated bioinformatics tools including UALCAN, GEO, LinkedOmics, GeneMANIA, STRING, CircNET and KOBAS.

Results Results indicated that ApoM is decreased in thyroid cancer tissues and is therefore negatively associated with malignant clinicopathological parameters. However, Kaplan-Meier analyses showed that ApoM expression is not an independent and significant prognostic factor for overall survival in thyroid cancer. LinkedOmics

Conclusions These results indicate that integrated bioinformatics analysis provide valuable information on apoM expression and potential regulatory networks in thyroid cancer. This information will be crucial in understanding the role of apoM in thyroid carcinogenesis.

Background

Thyroid cancer is the most common endocrine malignancy and presents in four differentiation types; anaplastic carcinoma, medullary carcinoma, follicular carcinoma and papillary carcinoma [1]. It is responsible for 567,233 cases worldwide, ranking in ninth place cancer incidences, or 1 in 20 cancer diagnoses in 2018 [2]. The estimated percentage of people who live at least five years after thyroid cancer diagnosis (5-year survival rate) is 98%. However, this rate varies depending on specific type, location, spread and stage of the disease. For instance, based on location and spread, the 5-year survival rate of localized, regional and metastatic thyroid cancer is 99%, 96% and 56%, respectively[3]. With the prevalence of unhealthy lifestyle and improvements in disease awareness and detection services, the incidence of thyroid cancer increased by 4.73 times from 2.40 per 100,000 in 2003 to 13.75 per 100,000 in 2012 with an average annual increase of 20%, while thyroid cancer mortality only increased slightly around 0.32 per 100,000 from 0.26 per 100,000 to 0.36 per 100,000 [4]. Therefore, it is necessary to identify reliable and effective biomarkers and unravel the genetic elements involved in thyroid carcinogenesis.

Apolipoprotein M (ApoM) is mainly associated with high-density lipoprotein (HDL), with only a small proportion located in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) [5]. Studies also indicate that ApoM plays a role in formation of pre β -HDL and in the antioxidative properties of HDL [6]. In addition, ApoM has been shown to play a role in stimulating HDL cholesterol efflux capacity and increasing its antioxidant activity [7, 8]. ApoM is a carrier and a regulator of sphingosine 1-phosphate (S1P), an important multi-functional bioactive lipid. ApoM also exerts its anti-oxidation and anti-inflammatory of HDL by delivering S1P to S1P receptors on endothelial cells [8-10]. Despite having

atheroprotective capacity, apoM have been shown to play a role in development of some cancers. Our group has previously explored the biological effects of apoM in liver cancer [11], colorectal cancer [12] and lung cancer [13]. We have reported the role of **ApoM in promoting proliferation and invasion in non-small cell lung cancers via upregulating sphingosine 1-phosphate receptor 1 (S1PR1) and activating the ERK1/2 and PI3K/AKT signaling pathways**[13]. However, the expression and role of ApoM in thyroid cancer development and progression is unclear.

In this study, we explored the role of ApoM in thyroid cancer, deducing biological insights from The Cancer Genome Atlas (TCGA) datasets and Gene Expression Omnibus (GEO) datasets. Using integrated bioinformatics, we analyzed the correlation, survival, Gene Ontology (GO) and relative gene expression of apoM in patients with thyroid cancer. So, we can obtain overall information regarding the function of apoM to uncover the underlying mechanisms of thyroid cancer occurrence and development.

Methods

UALCAN analysis

UALCAN (<http://ualcan.path.uab.edu/analysis.html>) is a comprehensive, user-friendly, and interactive web resource for analyzing cancer transcriptome data. The tool provides publicly accessible cancer transcriptome data (TCGA and MET500 transcriptome sequencing), with graphs and plots depicting gene expression and patient survival information. Using UALCAN, the effects of ApoM expression levels on relative clinicopathological parameters in TCGA thyroid cancer datasets were investigated.

GEO analysis

Gene expression data was downloaded from GEO database (<https://www.ncbi.nlm.nih.gov/gds>) with accession number GSE33630. This comprised of transcriptome of 11 anaplastic thyroid carcinomas, 49 papillary thyroid carcinomas and 45 normal thyroids hybridized onto Affymetrix Human Genome U133 Plus 2.0 arrays.

Gene ontology, function and correlation analysis

Gene Ontology (GO) analysis of ApoM was conducted with KOBAS v3.0 (<http://kobas.cbi.pku.edu.cn/>), a web server for gene/protein functional annotation and identification of gene set enrichment pathways [14]. High-throughput sequencing data (HiSeq RNA 01/28/2016) of TCGA_THCA were used for apoM. Correlation analyses were conducted using LinkedOmics (<http://www.linkedomics.org/>) at $p < 0.05$.

Potential interactions between apoM and different genes and circRNAs were predicted using the tools; GeneMANIA (<http://genemania.org/>), STRING (<http://string905.embl.de/>) and CircNet (<http://syslab5.nchu.edu.tw/CircNet/>).

Statistical analysis

Graphpad Prism 7.0 software (Graphpad Software Inc) was used for statistical analysis. Differential expression analysis of ApoM was conducted using LIMMA software package hosted by Bioconductor 3.1 at $p<0.05$.

Results

UALCAN analysis

From the TCGA thyroid cancer dataset, ApoM had a significantly higher expression in normal thyroid tissues than in tumor tissues (**Fig. 1**). Moreover, the associations between the ApoM mRNA expression levels and some clinicopathological features, including different tumor stages, histological subtype and nodal metastasis status, were present (**Fig. 2 A-C**). In survival analysis, the mRNA levels of apoM were not associated with overall survival in thyroid cancer (**Fig. 2D**). These findings suggest ApoM could play a potential tumor-suppressive role in thyroid cancer development and progression.

GEO data validation

As shown in Fig.3, GEO data (GSE33630) analysis showed that apoM mRNA expression was significantly higher in normal tissues than in thyroid cancer tissues ($P<0.001$) (**Fig. 3**).

Correlation and functional enrichment analysis

LinkedOmics dataset was used to screen the genes that were associated with apoM. From the Pearson correlation analysis, 19,927 genes were correlated with ApoM (**Fig. 4**). To expound the potential biological functions of apoM, GO analysis was performed in KOBAS 3.0. The GO function analysis showed that apoM was mainly enriched in regulation of lipoprotein and HDL metabolism, reverse cholesterol transport and protein-lipid complex assembly (**Fig. 5**).

Gene-gene, protein-protein and mRNA-circRNA network analysis

We constructed gene-gene and protein-protein interaction networks using web tools GeneMANIA and STRING, respectively. A two-layer model was constructed representing potential regulatory networks of ApoM in thyroid cancer. The inner layer represents ApoM gene while the outer layer comprises of genes that were co-expressed or genetically interacted with ApoM, including; KLF6, HGH1, TTR, AHSG, HNF4A, RAB17, SERPINA4, ARSE, KMO, RBP5, GLDC, APOA1, MSRA, CYP2J2, GCHFR, CCL16, RPAP1, WDR43, PLA2G12B, SLC22A7 (**Fig. 6A**). Proteins that are mainly interacted with ApoM are as following: apoA1, apoA2, PON1, FGB, apoC3, apoA4, apoB, apoC2, LPA, GPANK1 (**Fig. 6B**).

We queried the possible interaction between apoM and circRNAs using an online prediction tool, CircNet database. Analysis of ApoM mRNA-circRNA network yielded three circRNAs (circ-apoM-overlap.2, circ-apoM.1 and circ-apoM.6) interacting with apoM (**Fig. 6C**).

Discussion

This study investigated ApoM involvement in development of thyroid cancer using integrated bioinformatics tools. Integrated bioinformatics is mainly used for determining differential expression of target molecules and generating survival analyses. We report here downregulation of ApoM in thyroid cancer tissues compared to that of normal thyroid tissues. The expression level of ApoM mRNA was significantly associated with clinicopathological parameters from the TCGA database. There was no significant association between ApoM expression levels and survival of thyroid cancer patients. Using the GEO database, we further validated that ApoM mRNA levels were downregulated in the thyroid cancer tissues compared with the normal thyroid tissues. ApoM is predominately located in the cytosolic Golgi and plays an active role in lipoprotein metabolism and HDL assembly. Low levels of ApoM mRNA were observed in thyroid cancer tissues at late disease stage, nodal metastasis and histologic subtype. This implies that ApoM expression in normal tissues could affect normal physiological cellular function and inhibit malignant transformation.

ApoM mediates reverse cholesterol transport, plays an active role in formation of preβ-HDL and in the antioxidative properties of HDL [15]. However, the role of ApoM in cancer development and progression remains poorly understood. In our previous studies, ApoM enhanced proliferation and invasion in non-small cell lung cancers through upregulating S1PR1 and activating ERK1/2 and PI3K/AKT signaling pathways [13]. In another report, the levels of ApoM mRNA in colorectal cancer tissues were significantly increased in the patients with lymph node metastasis [12]. These findings are however contradictory to the results of the present study, necessitating further investigation on the role of ApoM in carcinogenesis.

Protein-protein interaction (PPI) networks and correlations analysis identified potential interactions between ApoM and other genes and circular RNA. ApoM and other apolipoproteins including ApoA1, ApoB and ApoC3, are main components of HDL. It was demonstrated that apoA-I can inhibit intestinal tumor growth and metastasis by promoting cholesterol efflux [16]. Moreover, D-4F, an apoA-I mimetic peptide, inhibits proliferation and tumorigenicity of epithelial ovarian cancer cells by upregulating the antioxidant enzyme MnSOD [17]. This implies that ApoM and other members of the apolipoprotein family (ApoA-I, ApoB and ApoAC3) could be regulated by the transcriptional factors KLF6 and HNF4, long circRNAs to inhibit cell proliferation and cell cycle in cancer development and progression. Studies have shown that some transcriptional factors including HNF1, LXRA, ER and Foxa2 can regulate ApoM gene expression [18-21].

From the GO enrichment results, ApoM was enriched in regulation of lipoprotein and HDL metabolism, reverse cholesterol transport and protein-lipid complex assembly. A recent study demonstrated that lack of ApoM in mice increases the amount of brown adipose tissue (BAT), accelerates clearance of postprandial triglycerides, and protects against diet-induced obesity. Moreover, ApoM-deficient mice phenotype is S1P-dependent and reflects diminished S1P1 stimulation [22]. In genetically modified mice, changes in plasma ApoM concentration resulted in quantitative and qualitative changes in HDLs, while its overexpression reduced atherosclerosis [6]. Furthermore, ApoM concentration is strongly correlated to total cholesterol in healthy individuals [23]. Clinical and experimental data have shown that hypercholesterolemia and a high-fat, high-cholesterol diet can affect cancer development. External

cholesterol can activate the oncogenic Hedgehog pathway, whereas internal cholesterol can induce mTORC1 signaling [24].

This study concludes that ApoM may serve as a diagnostic biomarker for patients with thyroid cancer. Using integrated bioinformatics, molecular pathways and ApoM-related molecules involved in thyroid cancer were identified. Further studies are required to validate putative ApoM functions and mechanisms of its involvement in thyroid cancer.

Declarations

Acknowledgments

Not applicable

Authors` contribution

Conceived and designed the experiments: JW NX GHL. Collected and analyzed the data: YY ZJ. Wrote the paper: JW YY NX GHL. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from TCGA database (<http://ualcan.path.uab.edu/analysis.html>), LinkedOmics database (<http://www.linkedomics.org/>) and GEO database (<http://www.ncbi.nlm.nih.gov/geo/>) with assessment number of GSE33630 ([https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi? acc= GSE33630](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33630)). Processed data are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

Abbreviations

Apolipoprotein M	apoM
High-density lipoprotein	HDL
Very low-density lipoprotein	VLDL
Sphingosine 1-phosphate	S1P
Sphingosine 1-phosphate receptor 1	S1PR1
Extracellular regulated protein kinases	ERK
Phosphatidylinositol 3-kinase	PI3K
Cancer Genome Atlas	TCGA
Gene Expression Omnibus	GEO
Gene Ontology	GO
Kruppel-like factor 6	KLF6
Protein HGH1 homolog	HGH1
Transthyretin	TTR
Alpha-2-HS-glycoprotein	AHSG
Hepatocyte Nuclear Factor 4 alpha	HNF4A
Ras-related protein Rab-17	RAB17
Kallikrein inhibitor 4	SERPINA4
Arylsulfatase E	ARSE
Kynurenine 3-monooxygenase	KMO
Retinol-binding protein 5	RBP5
Glycine cleavage system P protein	GLDC
Apolipoprotein A1	APOA1
Mitochondrial peptide methionine sulfoxide reductase	MSRA
Cytochrome P450 2J2	CYP2J2
GTP cyclohydrolase 1	GCHFR
C-C motif chemokine 16	CCL16
RNA polymerase II-associated protein 1	RPAP1
WD repeat-containing protein 43	WDR43
Phospholipase A2, group XIIB	PLA2G12B

Solute carrier family 22 member 7	SLC22A7
Apolipoprotein A2	ApoA2
paraoxonase/arylesterase 1	PON1
Fibrinogen beta chain	FGB
Apolipoprotein C3	ApoC3
Apolipoprotein B	apoB
Apolipoprotein C2	ApoC2
Lipoprotein A	LPA
G patch domain and ankyrin repeat-containing protein 1	GPANK1

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Figures

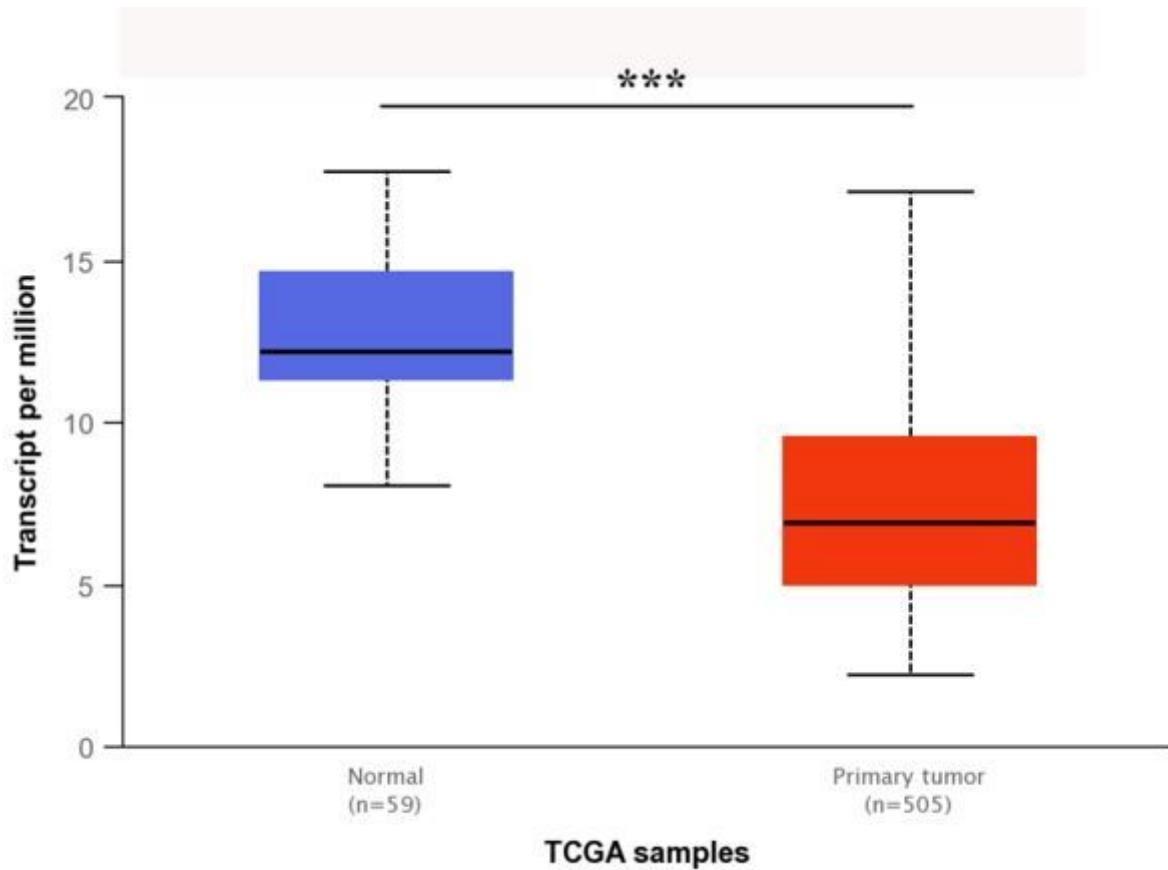


Figure 1

Downregulation of ApoM mRNA levels in thyroid cancer compared to normal thyroid tissues at *** $p<0.001$. Downregulation of apoM in thyroid cancer was confirmed with UALCAN dataset. *** P<0.001 vs normal thyroid tissues

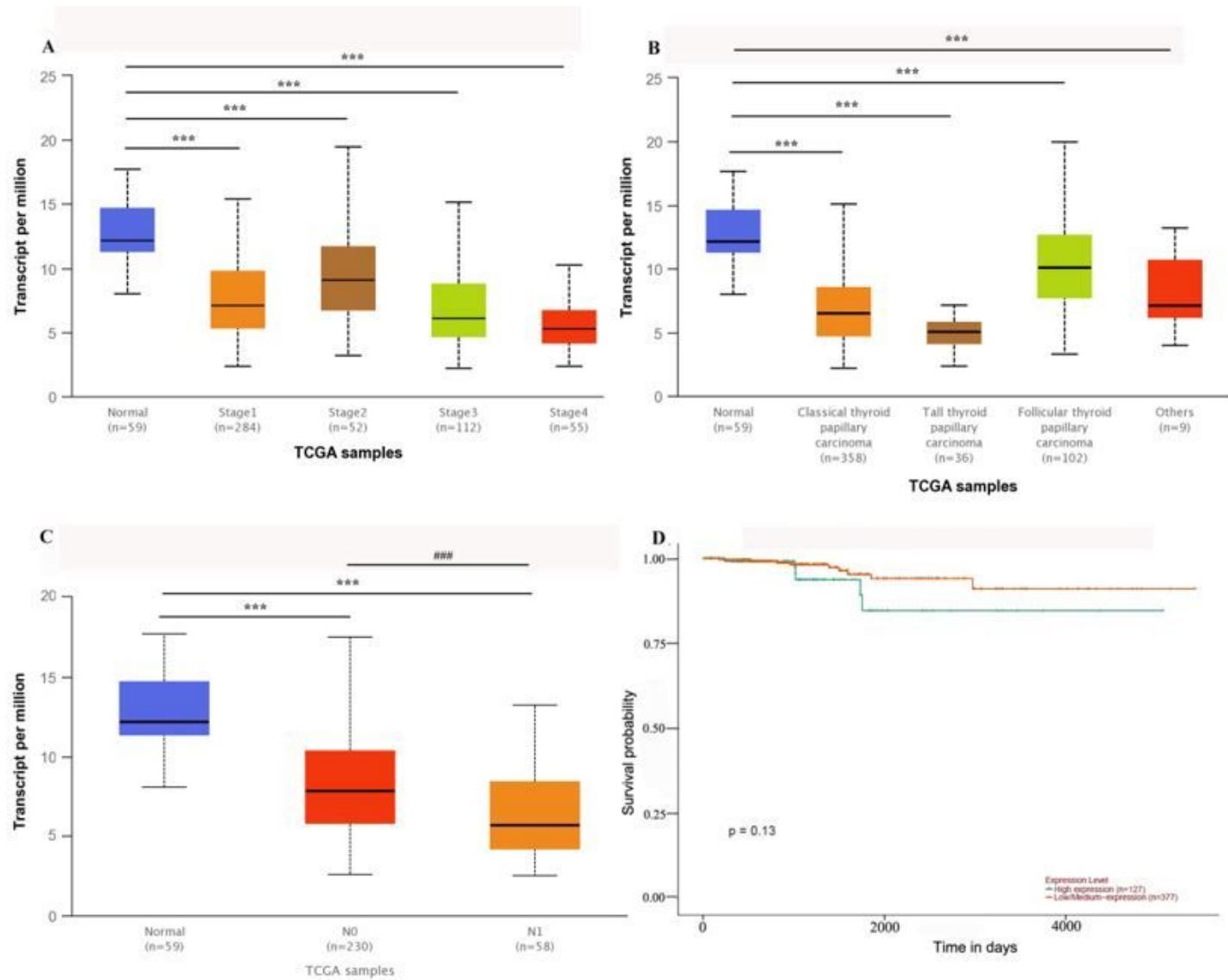


Figure 2

Association of ApoM expression with thyroid cancer: (A) pathological stages; (B) node metastasis and; (C) histology subtype. There was no significant difference in (D) survival time of patients at high, medium and low ApoM expression at ***p<0.001. *** P<0.001 vs normal thyroid tissues

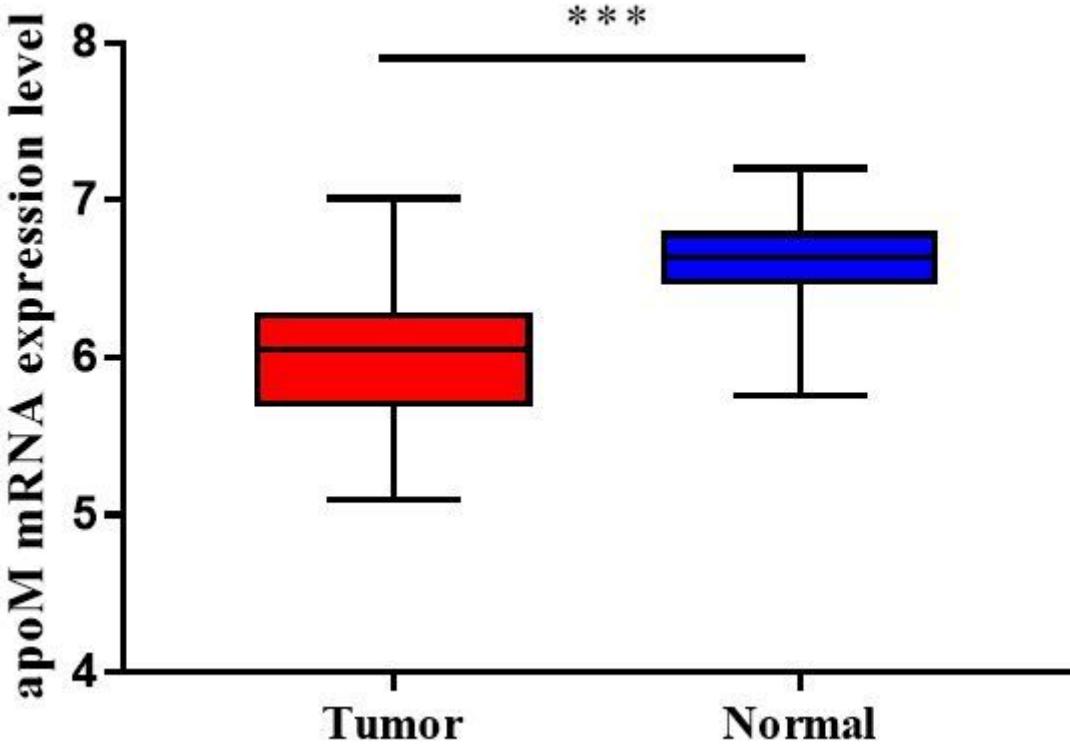


Figure 3

Expression levels of ApoM mRNA in thyroid cancer tissues and normal thyroid tissues at *** $p<0.001$. ***
 $P<0.001$ vs normal thyroid tissues

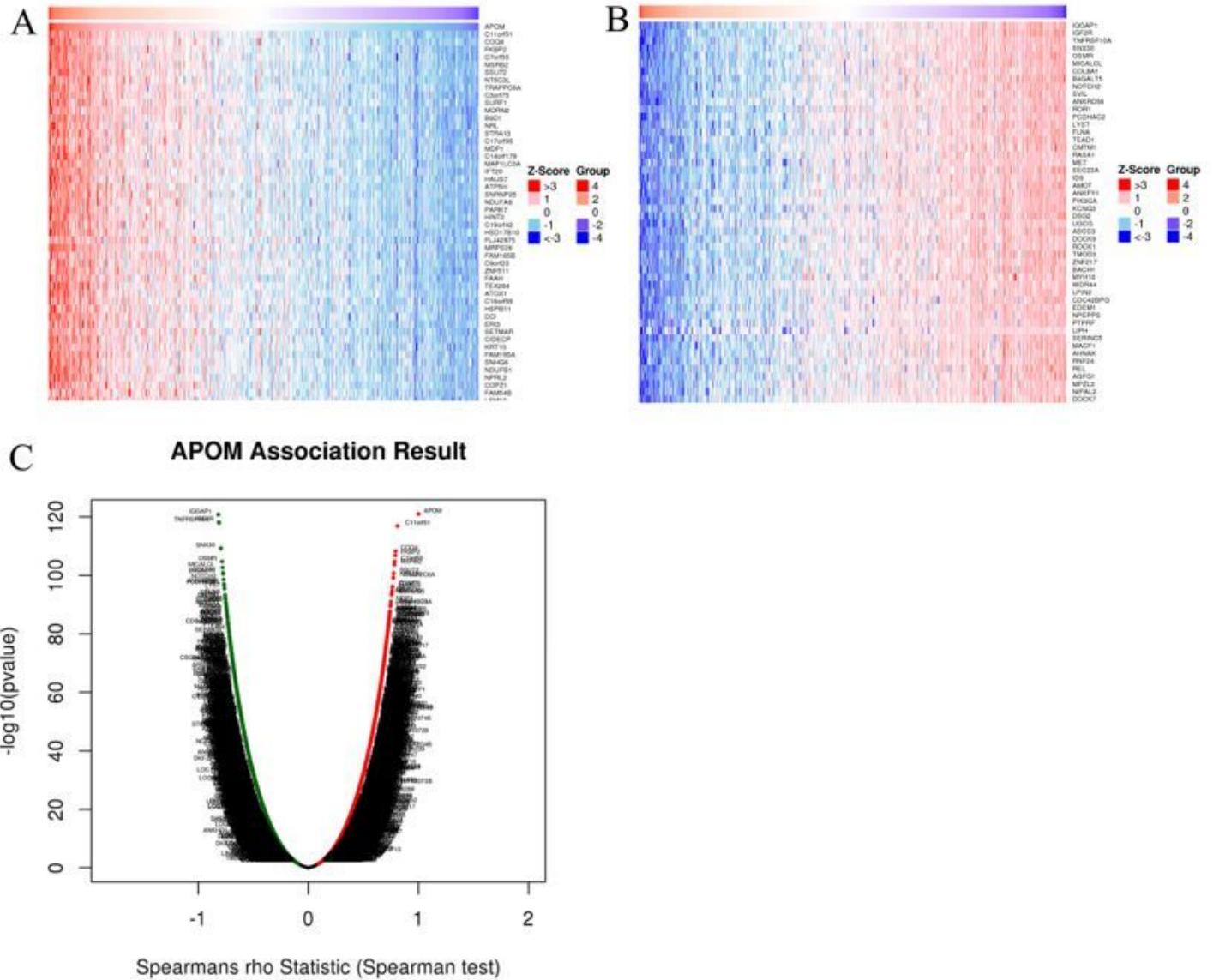


Figure 4

Correlation analysis ApoM in patients with thyroid cancer: (A) top 50 negatively related genes; (B) top 50 positively related genes; and (C) Pearson correlation of ApoM and its related genes.

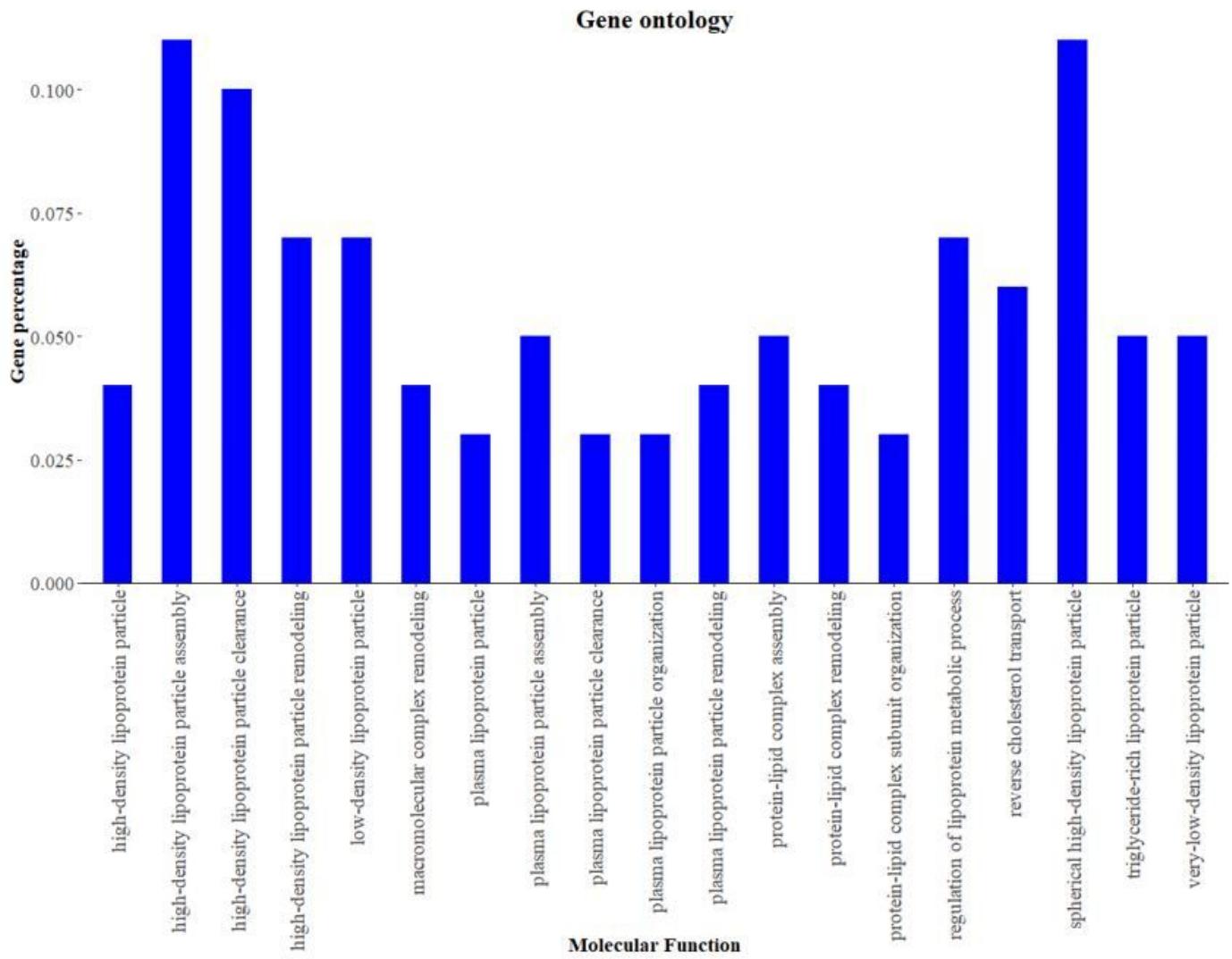


Figure 5

Gene Ontlogy (GO) enrichment analysis of ApoM

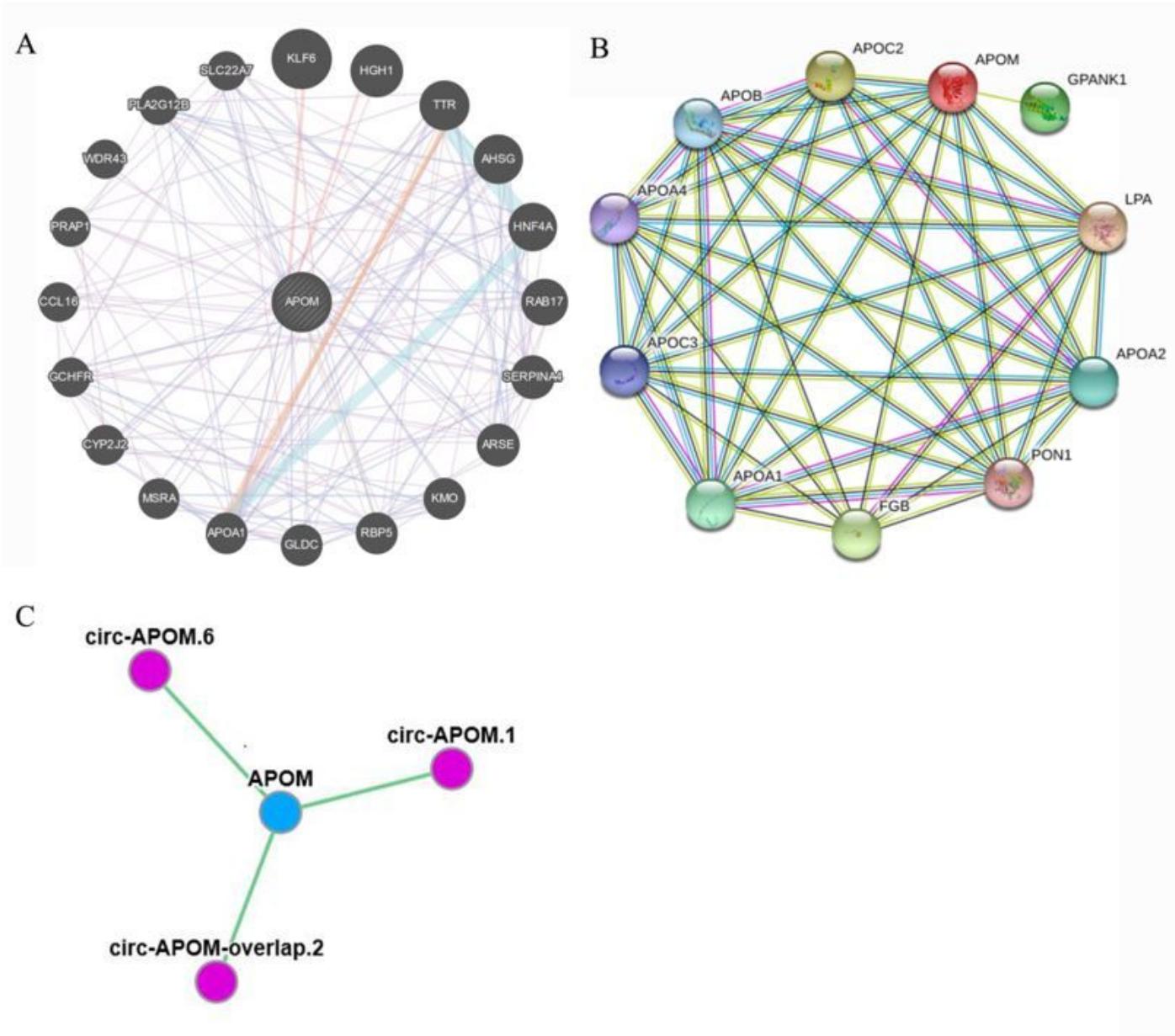


Figure 6

Gene-gene, protein-protein and mRNA-circRNA interaction networks: (A) Gene-gene interaction of apoM; (B) Protein-protein interaction network of apoM; and (C) ApoM-circRNA co-expression regulatory network.