Bioinformatic Analysis: Screening and Identification of Differentially Expressed Genes Modified by m6A in Ovarian Cancer

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Research article

Keywords: m6A, SCN7A, GATM, EIF4A3, FUS, Ovarian cancer

Posted Date: November 20th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-111366/v1>

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Abstract

Background: N6-methyladenosine(m6A) is one of the most common RNA modifications that occurs at the nitrogen-6 position of adenine. Emerging evidence has revealed that regulatory functions of m6A play an essential role in the development of cancer. However the study of m6A in ovarian cancer(OC) is still in our infancy. In this work ,we aimed to identify and analysis the differentially expressed genes(DEGs) modified by m6A which can provide new therapeutic targets and key biomarkers in OC.

Methods: We downloaded Microarray datasets GSE146553 and GSE124766 from Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) were identified by GEO2R analysis tools. Subsequently, The DAVID database was used to construct Enrichment analysis of GO and KEGG pathways. Next, the DEGs modified by m6A were identified by m6AVar database. Finally, the functional analysis and clinical sample validation of these genes were verified by ONCOMINE, GEPIA, cBioPortal online platform and Kaplan-Meier Plotter.

Results:152 DEGs were selected ,and the DEGs were mainly enriched in extracellular exosome, spindle microtubule, response to hypoxia and cell cycle .And we identified 15 DEGs which were modified by m6A:MAPK10, MXRA5, CHD7, MECOM, SCN7A, GREB, PRUNE2, MX2, TOP2A, JAM2, DST, LAPTM5, CDKN2A, GATM and ANGPTL1. After statistical analysis, two DEGs (SCN7A and GAMT) were selected for detailed study. We revealed that SCN7A and GAMT were expressed at a low level in OC. Afterwards, Survival analysis showed that SCN7A and GAMT expression were correlated with OC overall survival. And the expression of SCN7A and GAMT mRNA decreasing in different TNM stages. Finally, we presumed that the modification of m6A sponges GAMT via EIF4A3 or FUS to participate in the occurrence and the development of OC.

Conclusion: Altogether, the current study identified and analysed the DEGs modified by m6A in OC. It will help us to investigate the underlying mechanism and progression of OC. In addition, it can provide new diagnostic markers and potential therapeutic targets in OC.

1. Background

Ovarian cancer (OC) is the most lethal gynecological cancer and one of the leading causes of cancer-related death in women worldwide^[1, 2]. Aggressive frontline treatments with surgery and targeted chemotherapy are the main treatment of OC. However, due to late diagnosis, extensive metastasis and rapid development of chemotherapy resistance, the five-year survival rate of OC has not been improved^[3]. In order to reverse this situation, it is urgent to explore the molecular mechanism of ovarian cancer. Increasing evidences has revealed that abnormal gene expression and mutation play an important role in the pathogenesis of ovarian cancer. For example, overexpression of HOXC10 promotes the migration of ovarian cancer cells^[4]. In addition, H2AX is highly expressed in OC which can be used as a new prognostic biomarker for OC^[5]. Gene expression is regulated by various factors, including histone modification, DNA modification, RNA modification and so on. It has been recognized that epigenetic modification of DNA can regulate gene expression and chromatin tissue^[6]. Recently, an additional regulatory layer called "epitranscriptomics" has

been created, which depends on the chemical modification of RNA. Among them, N6-methyladenosine(m6A) is the most common internal modification of mRNA^[7, 8, 9, 10]. It refers to the methylation of adenosine base at position 6 of nitrogen. This methylation is a dynamically reversible modification that can be installed, removed and recognized by methyltransferases, demethylases and readers, respectively^[11]. According to their functions, they can be divided into three families(Fig. 1):"Eraser" protein (FTO and ALKBH5), which can remove the m6A modification from RNA^[12]."Reader" proteins (YTHDC1/2,YTHDF1/2/3,IGF2BP1 /2/3,hnRNPC,hnRNPG, etc).Their function is to specifically recognize and combine with the modified records of m6A^[13, 14, 15]; The main function of the last group of "Writer" proteins(METTL3, METTL14, WTAP, HAKAI, etc) is to catalyze the formation of m6A, which is a protein complex, named multicomponent m6A methyltransferase complex (MTC). METTL3 is the only catalytic subunit using S-adenosylmethionine (SAM) as a methyl donor^[18, 19, 20].It was reported that m6A modulator is a key participant in the malignant progression of hepatocellular carcinoma and a potential prognostic target^[21].Another study considered that m6A plays an important role in Gastrointestinal Cancer via PI3K/Akt and mTOR signaling pathway^[22]. Furthermore, high expression of m6A methyltransferase METTL3 can maintain tumorigenicity of colon cancer cells by inhibiting SOCS2^[23].However,the role of m6A in the development of OC needs further study, and the significance of its modified differential expression genes(DEGs) in OC needs to be explored.

Therefore,in this study we downloaded and analyzed two mRNA microarray data sets from Gene Expression Omnibus (GEO) to obtain DEGs between ovarian cancer and non cancer tissues. In order to help us understand the molecular mechanism of cancer occurrence and development, we also analyzed the KEGG and GO enrichment of DEGs. After that, we analyzed the genes modified by m6A in OC by m6Var database, crossed with the selected DEGs, selected two DEGs modified by m6A(SCN7A and GAMT), and analyzed these two candidate genes in detail. The combined analysis of m6A and SCN7A/GAMT will help us to explore the potential molecular mechanism of OC. It can also provide new diagnostic markers and therapeutic targets for the clinical treatment of OC.

2. Methods

Microarray data. GEO (<http://www.ncbi.nlm.nih.gov/geo>)^[24] is a common database for storing chips, second generation sequencing, and other high-throughput sequencing data.. Two gene expression datasets (GSE146553^[25]and GSE124766^[26]) were downloaded from GEO.By annotating the information, we first obtain the genetic symbol which converted from the probe.The GSE146553 dataset included 26 OC tissue samples and 3 normal samples. The GSE124766 included 8 OC samples and 3 normal samples.

2.1.Identification of DEGs.

GEO2R(<http://www.ncbi.nlm.nih.gov/geo/geo2r>) is an online analysis tool for GEO database, which can analyze differentially expressed genes in two or more sample datasets in GEO.The DEGs between OC and normal samples were screened using GEO2R.logFC (fold change) > 1.5 or LogFC<-1.5 and P-value < 0.01 were considered statistically significant.Furthermore, the online analysis tool of Wenn

chart(<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was used to obtain the DEGs contained in both data sets.

2.2.PPI network construction.

Search Tool for the Retrieval of Interacting Genes (STRING; <http://string-db.org>) (version 10.0)^[27] was used to predicted the PPI network. The purpose of this operation is to explore the functional interactions between proteins. Cytoscape (version 3.4.0)^[28] is a graphical display of the network software, but also for analysis and editing. It supports a variety of network description formats. Simultaneously, it can use the own editor module directly to build the network. The PPI networks and up/down regulation of the DEGs were drawn by Cytoscape.

2.3.KEGG and GO enrichment analyses of DEGs.

The Database for Annotation, Visualization and Integrated Discovery (DAVID; <http://david.ncifcrf.gov>)^[29] is a biometric database and free online analysis software. It integrates biological data and analytical tools to provide systematic and comprehensive biological functional annotation information for large-scale lists of genes or proteins to help us extract biological information from them. At present DAVID is mainly used for GO and KEGG pathway enrichment analysis.

KEGG^[30] (Kyoto encyclopedia of gene and genomes) is a comprehensive database that integrates genomic, chemical and system functional information. It enables us to understand advanced functions and biological systems, because it can obtain molecular level information through high-throughput experimental techniques, especially genome sequencing generated by large molecular data sets.

GO (Gene Ontology) is regarded as a key bioinformatics tool for annotating and classifying genes according to biology process, molecular function and cellular location^[31]. To analyze the function of DEGs, biological analyses were performed using DAVID online database. $P < 0.05$ was considered statistically significant.

2.4.Application of m6AVar database.

M6AVar database(<http://m6avar.renlab.org/index.html>)^[32] is a kind of database which records functional variants involved in m6A modification. It contains the site and specific location of m6A modification, related sequence, gene type, sample source, evidence confidence and so on. We performed m6AVar to identify the m6A modified DEGs and analyse the details of the modification process (modification sites, gene types, related binding proteins, etc).

2.5.Functional analysis and clinical sample certification of m6A-modified DEGs.

First, The relationship between expression patterns was analyzed using online database Oncomine (<http://www.oncomine.com>)^[33]. Sequentially, the overall survival analyses of m6A-modified DEGs were performed using Kaplan-Meier Plotter database(<https://kmplot.com/analysis/>)^[34]. And we also used Gen Expression Profiling Interactive Analysis (GEPIA; <http://gepia.cancer-pku.cn/>)^[35] to analyse expresssion by

stage. In addition, Overall survival and disease-free survival analyses of m6A-modified DEGs were also performed by cBioPortal online platform(<https://www.cbioportal.org/>)^[36]

3. Results

3.1. Identification of DEGs in OC and PPI network construction.

Two standard microarray datasets GSE146553 and GSE124766 were selected from GEO database and analyzed online by GEO2R respectively. A total of 689 and 970 DEGs were screened out. As shown in Venn Fig. 2: 152 DEGs overlapped in the two data sets. The function of genes in the body requires the interaction between genes or proteins which helps us to study the mechanism of the occurrence and development of diseases. So we built the PPI with STRING (Fig. 3A). In addition, we used the Cytoscape database to mark up and down regulated genes (Fig. 3B).

3.2. KEGG and GO enrichment analyses of DEGs.

We performed DAVID to finish the KEGG and GO enrichment analyses. GO Analysis is divided into three parts: biological processes (BP), molecular function (MF) and cell component (CC). The result showed that changes in BP of DEGs were mainly enriched activation of protein kinase activity, response to hypoxia and signal transduction (Table 1). Changes in MF were noticeably enriched in binding, structural molecule activity and protein homodimerization activity (Table 1). Changes in CC of DEGs were greatly enriched in extracellular exosome, spindle microtubule, membrane and mitotic spindle (Table 1). KEGG pathway analysis demonstrated that the downregulated DEGs were significantly enriched in tight junction and epithelial cell signaling in *Helicobacter pylori* infection, whereas the upregulated DEGs were mainly enriched in cell cycle and Oocyte meiosis (Table 1).

3.3. Selection of M6A modified DEGs

A total of 1219 m6A modified genes were screened from the m6AVar database. As illustrated Fig. 4 that 15 candidate m6A modified DEGs were obtained between m6A modified genes and GSE146553/GSE124766: MAPK10, MXRA5, CHD7, MECOM, SCN7A, GREB, PRUNE2, MX2, TOP2A, JAM2, DST, LAPTM5, CDKN2A, GATM and ANGPTL1. These candidate DEGs included 8 high expression genes and 7 low expression genes (Table.2). Subsequently, we analyzed these 15 candidate DEGs respectively through Kaplan-Meier Plotter database, GEPIA and cBioPortal ($P < 0.05$ was considered statistically significant) (Table.3). Finally, SCN7A and GATM are selected for further study.

3.4. Function analysis and clinical sample certification of two identified DEGs.

Firstly, Oncomine analysis of cancer and normal tissues showed that SCN7A and GATM were dramatically lower expressed in OC in different data sets (Fig. 5A-F). This result was also confirmed in GEPIA database (Fig. 5G, H). Afterwards, to accurately investigate the relationship between the survival time of OC patients and the expression levels of SCN7A and GATM, we analyzed the TCGA data of ovarian cancer by Kaplan-Meier Plotter database. The results suggested that the overall survival time (OS) of patients with low SCN7A

and GATM expression in OC was shorter than that in the high expression group (SCN7A $p = 0.0048$, GATM $p = 0.043$) (Fig. 6A,B). So we can see the expression levels of SCN7A and GATM had a significantly impact on the overall survival time of patients. Afterwards, we analyzed SCN7A and GATM by stages, GEPIA database analysis indicated that the expression of SCN7A and GATM was different in different TNM stages of OC, and the expression of SCN7A and GATM in stage II and III was higher than that in stage IV (Fig. 7A,B). Kaplan-Meier Plotter database explored that the expression levels of SCN7A and GATM could predict the prognosis of patients with stage I / II and III / IV (SCN7A p I-II = 0.01, SCN7A p III-IV = 0.0011, GATM p I-II = 0.0025, GATM p III-IV = 0.043) (Fig. 7C-F).

3.5. Analysis and hypothesis on the process of m6A modification

In order to further investigate the effect of m6A modified DEGs on the pathogenesis of OC, we analyzed the modification process in detail. We analyzed and sorted out the occurrence site, specific location, related binding protein, gene type, sample source and evidence credibility of m6A modification using m6AVar database (Table.4). These data imply that GATM has 11 (2 Mouse, 9 Human) research results, SCN7A has 94 (6 Mouse, 88 human) research results (due to the large number of SCN7A studies, the representative ones are selected for summary). The m6A modification of GATM mainly focused on chromosome 2, 15, 16. The related binding proteins were EIF4A3 and FUS. The former occurred mainly on the downstream of 3' AG, while the latter occurred on the upstream of 5' GT. It can be hypothesized that m6A silenced GATM via EIF4A3 or FUS to promote the occurrence and development of OC. The m6A modification of SCN7A mainly focuses on chromosome 2, whereas the protein pathway on which the m6A modification depended has not been documented.

4. Discussion

As one of the three major gynecological malignancies, ovarian cancer is a highly destructive and heterogeneous gynecological malignancy. Even with the development of many surgical techniques and chemotherapy, the overall five-year survival rate is still as low as 47%^[37]. Besides, OC is the fourth leading cause of cancer-related deaths in China^[38]. It is worth noting that the vast majority of ovarian cancer patients are in advanced stage when they are diagnosed, and the 5-year survival rate is less than 30%^[39]. These unsatisfactory data are mainly due to poor early diagnosis rate of OC^[40, 41] and poor prognosis^[42]. At present, the internationally recognized treatment for ovarian cancer is cytoreductive surgery, followed by platinum based chemotherapy. However, primary and secondary drug resistance may occur during chemotherapy of ovarian cancer, which makes it fail to achieve the desired effect^[43]. Recently, it was discussed that the unknown pathogenesis of OC is the main challenge to develop new diagnostic markers and therapeutic targets^[44]. This prompted us to further explore the molecular mechanism of ovarian cancer progression, in order to find valuable biomarkers and new treatment methods.

As one of the most common mRNA modifications, m6A is closely related to the development of human beings. RNA m6A methylation is a post transcriptional modification of adenine at position 6. This modification has been found in most eukaryotic mRNA, tRNA, rRNA and other noncoding RNAs. Its important regulatory functions include the regulation of gene expression, biological development and

cancer development. Especially the proliferation, apoptosis and metastasis of cancer. In breast cancer (BC), METTL3 ("Reader" protein of m6A modification) promotes HBXIP expression by increasing m6A modification, thus promoting BC proliferation^[45]. Another study on glioblastoma stem cells (GSC) showed that the m6A modification of METTL3 and METTL14 in GSC inhibited the expression of ADAM19, EPHA3 and other oncogenes, inhibited the growth and self-renewal of GSC, thus inhibiting tumorigenesis^[46]. In addition, it was reported that m6A modification also plays an important role in endometrial carcinoma (EC). High expression of METTL3 promotes the development of endometrioid epithelial ovarian cancer (EEOC) by regulating abnormal methylation of m6A RNA, indicating malignant tumor and low survival rate of patients^[47]. However, the study of m6A modification in ovarian cancer has only started in recent years. The fat mass and obesity associated protein (FTO) is a kind of m6A demethylase ("Eraser" protein of m6A modification). It can inhibit the self-renewal of ovarian tumor and cancer stem cells (CSC) and inhibit tumorigenesis in vivo, this process is completed by inhibiting cAMP signal transduction^[48]. Overexpression of TLR4 activates the NF - κ B pathway to upregulate the expression of ALKBH5 and increase the level of m6A, which promotes the occurrence of ovarian cancer. This discovery provides clues for the invention of new targeted therapy methods in OC^[49]. YTHDF1 ("Reader" proteins of m6A modification) can promote the occurrence and metastasis of ovarian cancer by binding with E6A modified EIF3C mRNA, and the up regulation of YTHDF1 is associated with poor prognosis of ovarian cancer patients^[50]. Another study showed that METTL3 plays a carcinogenic role partly through Akt signaling pathway in the process of esophageal cancer, which indicates that METTL3 can be used as a potential therapeutic target for esophageal cancer treatment^[51]. Thus, the above results demonstrated that m6A modification often depends on the related protein signaling pathway to abnormal expression of targeted genes, to participating in the proliferation, apoptosis and metastasis of cancer. Herein, the purpose of our study is to identify the differentially expressed genes (DEGs) modified by m6A in OC and investigate the protein pathway that the modification depends on.

In this study, two mRNA microarray data sets were analyzed to obtain DEG between OC and non-cancerous tissues. A total of 152 DEG were identified in two data sets, including 75 down-regulated genes and 77 up-regulated genes. GO and KEGG enrichment analysis was carried out to explore the interaction between DEGs. The over-expressed genes were mostly enriched in cell cycle, mitotic spin, activation of protein kinase activity, response to hydroxyia and oocyte meiosis, while the low-expressed genes were mostly enriched in signal transduction and protein domestication activity. It has been reported that the imbalance of cell cycle process and mitotic cell cycle play an important role in the occurrence or development of tumor^[52, 53, 54]. Furthermore, A large number of reports have suggested that complement activation is another way to promote tumor^[55]. In addition, hypoxia is the common result of rapid growth of solid tumors and abnormal vascular structure, which has been recognized as one of the most important characteristics of tumor microenvironment (hallmark)^[56]. More importantly, Hypoxia also induces the invasion of ovarian cancer to increase, and to chemoradiotherapy, including not sensitive or even resistant^[57, 58, 59, 60]. Consistent with these results, our results are consistent with all these theories.

Subsequently, we also screened 15 m6A-modified DEGs through the m6AVar database. The candidate genes were analyzed by Kaplan-Meier Plotter database, GEPIA and cBioPortal online platform, two DEGs were selected for detailed analysis: SCN7A and GATM. In different data sets, SCN7A and GATM were significantly low expressed in OC, and then the impact of the expression levels of the two genes on the prognosis of patients was studied. The results confirmed that the expression levels of SCN7A and GATM had a significant impact on the overall survival time of patients. Besides, the expression levels of SCN7A and GATM in OC of different TNM stages were different, and the expression of SCN7A and GATM in stage II and stage III was higher than that in stage IV. Mounting studies have shown that during the transcriptional regulation of BCL2 family and IAP family genes by ras-pi3k-akt-nf- κ B pathway, SCN7A is regulated and dramatically down regulated, leading to tumor proliferation and inhibiting tumor apoptosis^[61]. SCN7A as a BM related gene carrying frequent brain metastasis (BM) specific mutations. In colorectal cancer (CRC), SCN7A mutation leads to down-regulation of gene expression and promotes BM of CRC^[62]. It can be seen that our study on SCN7A is consistent with the above conclusion. GATM is a proximal tubular enzyme involved in creatine biosynthesis^[63]. In renal cell carcinoma (RCC), the expression of GATM RNA chimeras is increased, compared with benign adjacent kidneys, this up regulation of RNA chimeras may regulate the cellular mechanism and may affect the survival of patients^[64]. All these revealed that the role of SCN7A and GATM in OC needs further research and exploration.

The analysis of the methylation of the two genes was completed through the m6AVar database. The results included 11 GATM studies (2 mouse and 9 human) and 94 SCN7A studies (6 mouse and 88 human). The modification of SCN7A by m6A is mainly concentrated on chromosome 2, but the protein pathway dependent on m6A modification has not been recorded. On the other hand, the m6A modification of GATM mainly concentrated on chromosome 2, 15 and 16. The related binding proteins were EIF4A3 and FUS. EIF4A3 mainly occurred in the downstream of 3'AG and FUS occurred in the upstream of 5'GT. The present study aimed that CircPVRL3 can activate FUS, LIN28A, PTB and EIF4A3 binding proteins, promote the methylation of m6A, thus promoting the occurrence of gastric cancer (GC)^[65]. Linc00667 up-regulated in non-small cell lung cancer (NSCLC) can promote the production of vascular endothelial growth factor A (VEGFA) by binding with EIF4A3, thus promoting the proliferation, migration and angiogenesis of NSCLC cells^[66]. It has been previously shown that LncRNA EMX2OS binds directly to FUS protein, and overexpression of both can inhibit the proliferation, migration and invasion of prostate cancer cells and activate the GMP-PKG pathway^[67]. In the case, combined with the above information, we can assume that m6A can promote OC cell development by reducing GATM gene expression through EIF4A3 or FUS. Of course, this hypothesis needs to be further verified in future research.

Conclusion

The aim of this study was to identify the m6A modified DEGs that may be involved in the carcinogenesis or progression of OC. A total of 152 DEGs were identified, of which 15 were modified by m6A. SCN7A and GATM selected from them can be regarded as diagnostic biomarkers of OC. Finally, it is hypothesized that m6A may be a new therapeutic target for OC by reducing GATM gene expression through EIF4A3 or FUS

protein pathway. Whereas, in order to illuminate the biological function of these genes in OC, further research should be carried out.

Abbreviations

m6A: N6-methyladenosine

OC: ovarian cancer

DEGs: differentially expressed genes

GEO: Gene Expression Omnibus

MTC: methyltransferase complex

SAM: S-adenosylmethionine

STRING: Search Tool for the Retrieval of Interacting Genes

DAVID: The Database for Annotation, Visualization and Integrated Discovery

KEGG: Kyoto encyclopedia of gene and genomes

GO: Gene Ontology

GEPIA: Gen Expression Profiling Interactive Analysis

BP: biological processes

MF: molecular function

CC: cell component

OS: overall survival time

BC: breast cancer

GSC: glioblastoma stem cells

EC: endometrial carcinoma

EEOC: endometrioid epithelial ovarian cancer

FTO: fat mass and obesity associated protein

CSC: cancer stem cells

BM: brain metastasis

CRC: colorectal cancer

RCC: renal cell carcinoma

GC: gastric cancer

NSCLC: non-small cell lung cancer

VEGFA: vascular endothelial growth factor A

Declarations

Acknowledgement:

We thank Ge Lou for his guidance.

Funding section:

This work was partly funded by the National Natural Science Foundation of China (No. 81872507) and the HaiYan Foundation(No.JJZD2017-01).

Author contributions statement:

H-DL conceived the study, wrote the manuscript, and completed the figures and tables. G-L conceived the study and organized and edited the text. All authors have read and approved the manuscript.

Conflicts of interest:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that may be construed as a potential conflict of interest.

Ethics approval and consent to participate:

Not Applicable

Consent to publish:

Not Applicable

Availability of data and materials:

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests:

The authors declare that the research was conducted in the absence of any commercial or financial relationships that may be construed as a potential conflict of interest.

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Tables

Table 1
GO and KEGG pathway enrichment analysis of DEGs in OC samples.

Term	Description	Count in gene set	P-value
Upregulated	extracellular exosome	23	1.60E-04
GO:0070062			
GO:0005876	spindle microtubule	4	4.58E-04
GO:0016020	membrane	12	0.00178
GO:0005524	ATP binding	14	0.00784
GO:0032147	activation of protein kinase activity	3	0.00242
GO:0005198	structural molecule activity	5	0.00324
GO:0001666	response to hypoxia	4	0.00479
GO:0072686	mitotic spindle	3	0.00942
Cfa04114	Oocyte meiosis	5	0.00276
Cfa04110	Cell cycle	8	3.68E-06
Downregulated	signal transduction	12	0.00213
GO:0007165			
GO:0042803	protein homodimerization activity	9	0.00557
GO:0021762	substantia nigra development	3	0.01284
GO:1903779	regulation of cardiac conduction	3	0.01655
GO:0002318	myeloid progenitor cell differentiation	2	0.02434
GO:0009791	post-embryonic development	3	0.02719
GO:0006816	calcium ion transport	3	0.02929
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	3	0.01742
hsa04530	Tight junction	3	0.02842

Table 2. Functional roles of 4 DEGs modified by m6A
(Upregulated genes are marked in red; downregulated genes are marked in black)

No.	Gene Symbol	Full Name	Function
1	CHD7	Chromodomain-helicase-DNA-binding protein 7	It promotes the production of 45S precursor rRNA and plays an important regulatory role in the transcription process ^[68] .
2	SCN7A	Sodium channel protein type 7 subunit alpha	Regulate the permeability of the sodium ion excitation membrane which is voltage dependent.
3	MX2	Interferon-induced binding protein Mx2	GTP-MX2 has strong antiviral activity, especially against HIV-1 and SIV-mnd. It is also involved in the development of nucleocytoplasmic transport and cell cycle ^[69,70] .
4	GATM	Glycine amidinotransferase, mitochondrial	Promoting the synthesis of creatine, which is involved in embryonic and the process of central nervous system, may also participate in the progression of heart failure.
5	MAPK10	Mitogen-activated protein kinase 10	MAPK10 regulates multiple processes of nerve cell progression, including proliferation, differentiation, apoptosis, etc ^[71] .
6	MXRA5	Matrix-remodeling-associated protein 5	It plays an anti-inflammatory and anti-fibrotic role through regulating TGF β 1 and pro-inflammatory stimuli ^[72] .
7	ANGPTL1	Angiopoietin-related protein 1	Because the protein it encodes is a member of the angiogenin family, it has higher mRNA levels in highly vascularized tissues.
8	MECOM	Histone-lysine methyltransferase MECOM	MECOM is a transcriptional regulator that can regulate the DNA sequence in the promoter region of a target gene.
9	GREB1	GREB1-like protein	Plays a major role in early metanephros and genital development
10	PRUNE2	Protein prune homolog 2	It is involved in the survival, invasion and differentiation of tumor cells.
11	TOP2A	DNA topoisomerase 2-alpha	The ARNTL/BMAL1 transcriptional oscillation process is involved by regulating the topological state of DNA, thus changing its period length ^[73] .
12	JAM2	Junctional adhesion molecule B	Junctional adhesion protein that mediates heterotypic cell-cell interactions with its cognate receptor JAM3 to regulate different cellular processes
13	DST	Dystonin	As a protein integrator, it is involved in the connection of actin, intermediate filaments and microtubule cytoskeletal networks.
14	LAPTM5	Lysosomal-associated transmembrane protein 5	It plays a special regulatory role in adult hematopoietic cells and embryogenesis.
15	CDKN2A	Cyclin-dependent inhibitor 2A	kinaseNegative regulation of normal cell proliferation process.

Table 3

Analysis statistics of m6A modified DEGs(OS:Overall survival PFS:disease-free survival YES:There was statistical significance NO:There was no statistical significance)

No.	Gene	OS Kaplan-Meier	OS by Stage (Kaplan-Meier)	OS (GEPIA)	Stage analysis (GEPIA)	OS (CBioPortal)	PFS (CBioPortal)
1	CHD7	NO	NO	NO	YES	NO	NO
2	SCN7A	YES	YES	YES	YES	NO	NO
3	MX2	YES	NO	NO	YES	NO	NO
4	GATM	YES	YES	YES	YES	NO	NO
5	MAPK10	YES	YES	NO	NO	NO	NO
6	MXRA5	YES	NO	NO	NO	YES	NO
7	ANGPTL1	YES	NO	NO	NO	NO	NO
8	MECOM	NO	NO	NO	NO	NO	NO
9	GREB1	YES	YES	NO	YES	NO	NO
10	PRUNE2	NO	NO	NO	NO	NO	NO
11	TOP2A	YES	YES	NO	NO	NO	NO
12	JAM2	YES	YES	NO	NO	NO	NO
13	DST	NO	NO	NO	NO	NO	NO
14	LAPTM5	NO	NO	NO	NO	NO	NO
15	CDKN2A	YES	YES	NO	NO	NO	NO

Table 4
Summary of the modification process of m6A.(RBP:related binding protein)

Gene: GAMT							
M6A-ID	Species	Chromosome	Gene type	RBP	m6A(source)	Splicing Site	Relative position
405526	Human	Chr16	Protein coding	EIF4A3	Prediction(Low)	3'-AG	Downstream 8 bp
283865	Human	Chr15	Protein coding	EIF4A3	Prediction(Low)	3'-AG	Downstream 36 bp
18403	Mouse	Chr2	Protein coding		MeRIP-Seq(Medium)	3'-AG	Downstream 92 bp
283862	Human	Chr15	Protein coding	FUS	Prediction(Low)	5'-GT	Upstream 71 bp
283863	Human	Chr15	Protein coding	FUS	Prediction(Low)	5'-GT	Upstream 73 bp
283864	Human	Chr15	Protein coding	FUS	Prediction(Low)	3'-AG	Downstream 76 bp
405526	Human	Chr15	Protein coding	EIF4A3	Prediction(Low)	3'-AG	Downstream 8 bp
84153	Mouse	Chr2	Protein coding	EIF4A3	Prediction(Low)		
385902	Human	Chr15	Protein coding		Prediction(Low)		
283868	Human	Chr15	Protein coding		Prediction(Low)		
283867	Human	Chr15	Protein coding		Prediction(Low)		
283866	Human	Chr15	Protein coding		Prediction(Low)		
Gene: SCN7A							
M6A-ID	Species	Chromosome	Gene type	RBP	m6A(source)	Splicing Site	Relative position
83345	Mouse	Chr2	Protein coding		Prediction(Low)	5'-GT	Upstream 82 bp
146083	Human	Chr2	Protein coding		Prediction(Low)	3'-AG	Downstream 2 bp
146108	Human	Chr2	Protein coding		Prediction(Low)	5'-GT	Upstream 71 bp

Gene: GAMT						
146112	Human	Chr2	Protein coding	Prediction(Low)	5'-GT 3'-AG	Upstream 57 bp Downstream 14 bp
146122	Human	Chr2	Protein coding	Prediction(Low)	3'-AG	Downstream 93 bp
377247	Human	Chr2	Protein coding	Prediction(Low)	3'-AG 5'-GT	Downstream 56 bp Upstream 37 bp

Figures

Figure 1

The brief introduction of m6A

GSE146553

Figure 2

DEGs selected from GSE146553 and GSE124766 Venn diagram: DEGs were selected with a fold change >1.5 / <-1.5 and P-value <0.01 among the gene expression profiling sets GSE146553 and GSE124766. The 2 datasets showed an overlap of 152 genes

Figure 3

The PPI network of DEGs A. The PPI network of DEGs was performed by STRING B. The PPI network of DEGs was performed by Cytoscape (High expression genes are marked in red and low expression genes are marked in blue.)

Figure 4

DEGs selected from GSE146553 , GSE124766 and m6A modified DEGs. (Venn diagram: the 3 datasets showed an overlap of 15 genes.)

Figure 5

Expression of SCN7A and GATM between normal tissues and ovarian cancer tissues in 3 studies identified in the Oncomine database and the GEPIA database.(A,D:Ovarian Serous Carcinoma vs. Normal in Welsh Ovarian Statistics[74]. B,E: Ovarian Serous Adenocarcinoma vs. Normal in Adib Ovarian Statistics[75]. C,F: Ovarian Serous Adenocarcinoma vs. Normal in Yoshihara Ovarian Statistics. G,H:the GEPIA database)

Figure 6

Relationship between the expression of SCN7A/GATM and the prognosis of OC

Figure 7

Relationship between the expression of SCN7A/GATM and the differet TNM grades in OC.(A,B:the GEPIA database;C-F:Kaplan-Meier Plotter database)