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Research

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CPT1B, a metabolic molecule, is also an independent risk factor in CN-AML

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0 Abstract:

Background: In recent years, fatty acid oxidation has been considered as an important energy source for tumorigenesis and development. There are extensive studies on CPT1A, a kind of fatty acid oxidation rate-limiting enzyme. However, prognostic value and regulatory network of another subtype CPT1B in AML remains elusive. This submission aims to clarify the independent prognostic role of the metabolic molecule CPT1B in CN-AML from clinical data and molecule levels including mRNA, miRNA and lncRNA. **Method:** First, we analysed the CPT1B expression in AML cohort via online database “GEPIA”. Following the assessments on differential mRNA, miRNA, lncRNA expression analysis on the paired CPT1B high and low expression groups, there were abnormal changes at different molecular levels. Moreover, we determine the preliminary predictions on the regulatory network of CPT1B in AML by constructing miRNA-mRNA and ceRNA networks. The study had yielded a series of molecules that potentially proved the prognostic prediction and metabolic function of CPT1B from the side. Finally, we analysed the CPT1B expression in our own cohort to make a verification. **Result:** Notably, there was a significantly high expression of CPT1B in AML patients than normal people and was associated with the poor outcome. Molecules from different levels verified our finding and revealed the possible regulatory mechanism of CPT1B in AML. **Conclusion:** CPT1B is a potential prognostic factor as well as a therapeutic target of AML.

Key Words:

CPT1B, prognostic factor, differential molecules, miRNA-mRNA network, ceRNA network

1 Background:

Acute myelocytic leukemia (AML), a group of hematological malignancies, is associated with high heterogeneity in terms of cell morphology, molecular biology, immunotyping, and cytogenetics. This disease is characterized by high mortality, high treatment-related mortality, high relapse rates and poor clinical outcomes [1]. Though poor results are partly attributed to the

shortage of molecular markers for personalized therapy, currently, much of the studies efforts have been concentrated on finding effective prognostic biomarkers. Elsewhere, chromosomal abnormalities were shown as an effective tool for AML risk stratification but cytogenetically normal acute myeloid leukemia (CN-AML) accounts for 50% of total AML and constitutes the main body of intermediate-risk AML with heterogeneous groups [2]. In CN-AML patients risk stratification, molecular diagnostics of NPM1, FLT3-ITD and CEBPA mutation analysis are essential [3]. Since CN-AML revealed specific cytogenetic consistency and without abnormalities in their chromosomes, they provide a perfect platform in determining AML biomarkers. Besides, identification of molecular signals such as DNA mutations, abnormal expression of mRNA and miRNA can be favourable or unfavorable biomarkers.

Based on studies, changes in metabolic signals were related to the occurrence of leukemia, hence offers potential insights in developing AML drug treatments [4]. Warburg effect of aerobic glycolysis is considered to be an important source of bioenergy in the past, which can promote rapid cell proliferation [5]. But nowadays, people put more and more attention to other effects, especially fatty acid oxidation (FAO) [6], which is regarded as playing important roles in the cancer pathogenesis. The carnitine palmitoyltransferase 1A (CPT1A), a subtype of CPT1, potentially catalyzes the rate-limiting step in FAO. Moreover, it has been established as an independent risk factor in AML poor prognosis [7]. What's more, Ricciardi MR et al have discussed the function of CPT1A inhibitor (ST1326) in blocking FAO and its inherent mechanism about in vitro antileukemic activity on leukemia cell lines and primary cells obtained from patients with hematologic malignancies [8]. But the prognosis role and regulatory mechanism of another subtype of CPT1 named CPT1B in AML remains elusive. In addition, studies on CPT1B were limited to fatty acid metabolism but a few on tumors. Previous studies had determined STAT3-CPT1B-FAO Pathway promotes breast cancer progress and its chemoresistance [9], hence revealed the potential of CPT1B in prognosis and therapeutic targets.

Taken together, these results highlight the potential carcinogenicity of CPT1B in AML. However, the clinical relevance of CPT1B and AML as well as the possible upstream and downstream regulatory mechanisms of CPT1B in AML remain unclear.

Herein, we presented CPT1B as a prognostic biomarker for AML with deep data showing association of high CPT1B expression with poor AML survival. The identified unique mRNA, miRNA, lncRNA patterns consequently decipher the biologic insights of high CPT1B expressors. Compared with existing experiments on CPT1B, we clarified the effect and mechanism of CPT1B in AML via bioinformatics and clinical data, which may give us new ideas on the treatment of AML especially CN-AML.

2 methods:

2.1 Clinical patients

The AML patients involved in this study were all diagnosed at the age more than 14. Bone marrow specimens for genetic analysis were collected from each patient at the time of diagnosis prior to any treatment. Clinical data of 325 AML patients were abstracted from medical records between January 2010 and July 2016. As previously described, we conducted WHO classification, cytogenetic and molecular analysis centrally at the Zhejiang Institute of Hematology (ZIH) in China [10]. Using methods as previously described, chromosomal abnormalities were analyzed and RT-qPCR were used to assess gene mutations included DNMT3A, NPM1, CEBPA, FLT3-ITD,

IDH1 and IDH2 [11]. The above cytogenetics analysis were performed by researchers without knowing the CPT1B expression level and clinical outcome. All of the patients participated in this study has provided written informed consent. This study was approved by the Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

2. 2 Quantitative Reverse Transcriptase-PCR

The process of RNA extraction, reverse transcription and quantitative PCR has been reported [12]. Quantification was based on $\Delta\Delta CT$ calculations and normalized to the expression of GAPDH as the loading control. PCR reactions were performed in a total volume of 10 μ l which contains 3.5 μ l of ddH₂O, 5 μ l of 2 $\times$ PCR Mix, 0.25 μ l of 0.5 μ M of each primer and 1 μ l of 100ng/ μ l sample cDNA. mRNA levels were normalized to GAPDH house keeping gene. The following primers were used for quantitative PCR:

CPT1B, 5'-CCTGCTACATGGCAACTGCTA-3' (sense) and
5'-AGAGGTGCCCAATGATGGA-3' (antisense)
GAPDH (control), 5'-GGAGCGAGATCCCTCCAAAAT-3' (sense) and
5'-GGCTGTTGTCATACTTCTCATGG-3' (antisense).

2. 3 Statistical Analysis

2. 3. 1 Clinical data analysis

Patient characteristics were summarized by descriptive statistics, including frequency counts, median, and range. The main purpose of this study was to clarify the prognostic value of CPT1B expression in AML patients as well as the possible upstream and downstream regulatory molecules and pathways of CPT1B in AML. Overall survival (OS) is defined as the time from the date of diagnosis to death due to any causes. The definition of event free survival (EFS) was time from date of diagnosis to removal from the study because of the absence of complete remission (CR), relapse or death. Adjusted variables such as age, WBC, DNMT3A gene, IDH1 and IDH2 mutations were considered to be recognized indicators for AML patients. The association between CPT1B expression and OS, EFS was assessed by the Kaplan-Meier method and log-rank test. The Fisher exact and Wilcoxon rank-sum tests were used, respectively for categorical and continuous variables, so as to assess the association between expression levels and clinical molecular characteristics. The impacts of CPT1B expression to OS and EFS were evaluated via multivariable hazards models in the presence of other known risk factors.

2. 3. 2 Sequencing Data Analyses

Expression profiles of mRNAs, miRNAs, lncRNAs and clinical information about 68 CN-AML patients were obtained by high throughput sequencing (RNA-Seq), derived from the Cancer Genome Atlas (TCGA) (<https://tcga-data.nci.nih.gov/>). The sequenced data was obtained from Illumina HiSeq RNASeq and Illumina HiSeq miRNA-Seq platforms. Our research complied with the publication guidelines provided by TCGA (<http://cancergenome.nih.gov/publications/publication-guidelines>). For RNA-Seq data, expression levels of mRNAs, miRNAs and lncRNAs were calculated as RPKM and RPM (Reads Per Kilo-base per Million reads). To control the influence of other factors, we matched each CN-AML patient with high and low CPT1B expression based on variables like age, sex, karyotype, WBC, and genes of FLT3-ITD, NPM1, CEBPA, DNMT3A among TCGA cohort, which ultimately helped us get 10 pairs. As for finding

differential mRNAs, miRNAs, lncRNAs between these 10 pairs, we selected the differential part of each pair with the criterion of $FC \geq 1.5$, respectively. Then we intersected the differential parts of these ten matched pairs, finally picked up the molecules which appears no less than two thirds of ten pairs for mRNAs and lncRNAs as well as no less than half of ten pairs for miRNAs as our final differential contents.

2. 3. 3 mRNA analysis

Protein protein interaction network (PPI network) was developed using STRING [13] and visualized by Cytoscape software [14]. The top30 hub genes analysis was done by the APP named “cytohubba” [15]. Hallmarks analysis was done by Gene Set Enrichment Analysis[16]. The data of the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was obtained from STRING before visualized via Cytoscape software.

2. 3. 4 miRNA analysis

The KEGG pathways enrichment for those DE miRNAs were performed via mirPath v3 [17]. Interaction of miRNAs and mRNAs integrative analysis were done by WGCNA via R statistic packages, version3.6.3 [18].

2. 3. 5 ceRNA network construction

The co-expression network of differentially expressed mRNAs, miRNAs and lncRNAs were constructed to ascertain the roles of mRNAs, miRNAs and lncRNAs in mediated ceRNA network. DE miRNAs targeting mRNAs were retrieved from miRTarBase (<http://mirtarbase.mbc.nctu.edu.tw/>) before verified on an experimental study using reporter assay, qRT-PCR, Western blot, microarray, and next-generation sequencing in miRTarBase. Besides, DE miRNAs targeting lncRNAs were obtained from miRcode (<http://www.mircode.org/>). Reliability of ceRNA network was improved by intersecting mRNAs and lncRNAs targeted by differential miRNAs with the differential mRNAs and lncRNAs analysed from the original TCGA data respectively. Consequently, the ceRNA network was visualized by Cytoscape v3.7.1 software.

3 Text:

3. 1. Association of CPT1B Overexpression with Poor Clinical Outcome in TCGA Cohort

With the analysis of public data sets in the GEPIA database, we explored the potential of CPT1B as an independent prognostic factor. CPT1B expression was associated with poor OS [HR: 2.6, $P = 0.001$] among 106 AML patients (Fig. 1A). Interestingly, CPT1B expression in AML patients was significantly higher than in their normal counterparts (Fig. 1B). These preliminary findings lead to subsequent experiments and data analysis.

3. 2. Genome-wide Expression Profiles and CPT1B Expression Associations

In the TCGA cohort, a meta-analysis of gene expression profiles was conducted in 68 CN-AML patients to explore the biological role of CPT1B in leukemogenesis. A total of 10 pairs were obtained by matching CN-AML patients with high and low CPT1B expression based on variables like age, sex, karyotype, WBC and genes including FLT3-ITD, NPM1, CEBPA, DNMT3A in TCGA cohort (Table S1). The 10 pairs were further subjected to differential analysis. The results showed a total of 413 up-regulated and 164 down-regulated genes in the TCGA cohort

(Table S2). The constructed PPI network via online data STRING (Table S3) was used to determine the interaction between proteins. Then we analysed the top30 hub genes via cytoHubba respectively in both up-regulated and down-regulated differential genes (Fig. 2A & B, Table S4).

In the TCGA data, differential pathways between CPT1B high and low expressors were analyzed using the GSEA method. There were upregulated hallmarks in CPT1B high expressors such as inflammatory response, P53 pathway, TNFA signaling via NFkB, fatty acid metabolism, IL6-JAK-STAT3, IL2-STAT5, KRAS signaling, and others (Fig. 2C). Most of these pathways were reported in AML. KEGG pathways enrichment analysis was particularly for the hub genes to elect the top30 pathways among the up-regulated and down-regulated hub genes, respectively (Fig. 2D & E). In the up-regulated hub genes, there were several pathways such as acute myeloid leukemia, Wnt signaling pathway, mTOR signaling pathway and so on whereas the down-regulated hub genes had NF-KB signaling, MAPK signaling and others.

According to KEGG results of hub genes, LEF1 (lymphoid enhancer-binding factor 1) was elected since its high expression serves as a novel favorable prognostic factor in cytogenetically normal acute myeloid leukemia [19]. However, ALAS2, an erythroid-specific mitochondrially located enzyme, potentially predict the OS outcome in AML [20]. Therefore, their genes and associated pathways can provide enormous knowledge of CPT1B.

3. 3. miRNA Expression Changes in High CPT1B Expression

The aforementioned criteria for matching the CN-AML patients with high and low CPT1B expression in the TCGA cohort (Table S1) was adopted whereby the differential miRNAs were further evaluated based on 10 pairs obtained. Consequently, 48 downregulated and 82 upregulated miRNAs in high CPT1B expressors (Table S5) were identified.

KEGG pathways enrichment for DE miRNAs via mirPath v3 were developed to explore their related pathways. The identified pathways were fatty acid metabolism, fatty acid elongation, citrate cycle (TCA cycle), cell cycle, p53 signaling pathway, TNF signaling pathway, mTOR signaling pathway, chronic myeloid leukemia, acute myeloid leukemia, etc for the up-regulated miRNAs. However, the down-regulated miRNAs had TGF- β pathway, TNF signaling pathway, PI3K-Akt signaling pathway, fatty acid biosynthesis, etc. (Fig. 3A & B, Table S6).

The results of KEGG pathways enrichment for DE mRNAs and DE miRNAs pointed out a certain direction for our subsequent research. By combining the KEGG results of miRNAs and mRNAs, we revealed that the role of CPT1B was likely related to P53, TNF, mTOR signaling pathway and several other metabolism processes.

3. 4. Integrative Analysis of mRNA and miRNA Interaction

An integrated analysis of mRNA and miRNA interaction in high and low expressors were conducted to determine their regulatory mechanism via WGCNA (abline picks 0.6). Five downregulated genes in high CPT1B expressors were predicted to be targeted by the upregulated miR-509.3. Moreover, 2 downregulated genes were likely to be targeted by upregulated miR-339 (Fig. 4A, Table S7).

Thereafter, the biological process and KEGG pathways enrichment analysis of dysregulated mRNAs and miRNAs in the miRNA-mRNA network were made. In silico analysis of BP process, miR-509.3 potentially regulate FST and MLF1 genes in the hematopoietic progenitor cell differentiation as well as HPN and SULF1 genes in the negative regulation of epithelial cell

proliferation and regulate BOLL in the positive regulation of translational initiation process. Besides, miR-455 regulate ACOT12 gene in acetyl-CoA metabolic process (Table S8). In KEGG pathways enrichment, miR-509.3 regulate FST in the TGF-beta signaling pathway whereas miR-339 can regulate both HIST2H2AA4 and HIST1H2AE in the necroptosis. Moreover, miR-455 regulate ACOT12 gene in pyruvate metabolism (Table S9). This analysis implicates some novel miRNA-mRNA regulated biological processes in high CPT1B expressors. Among the miRNAs, miR-339 was highly related to the prognosis of AML while the high expression of miR-339 was associated with poor survival of AML (Fig. 4B). Furthermore, ACOT12 (acyl-CoA thioesterase 12) was closely linked to the metabolism process which indicates the metabolic function of CPT1B.

3. 5. ceRNA network

Long non-coding RNAs (lncRNAs) have a considerable influence on tumorigenesis. To investigate the function of lncRNAs in different CPT1B expressors, we conducted the meta-analysis on lncRNA expression profiles of 68 CN-AML patients based on the TCGA cohort. Patients matching and their analysis were conducted as aforementioned (Table S1). From the analysis, 116 up-regulated and 59 down-regulated lncRNAs were identified (Table S10).

The lncRNAs were predicted using differential miRNAs based on online database LncBase-v2 before intersected with the DE lncRNAs that was earlier analyzed. In this process, we identified 28 up-regulated miRNAs and 14 down-regulated lncRNAs as well as 15 down-regulated miRNAs and 10 up-regulated lncRNAs (Table S11). The 28 up-regulated miRNAs and 15 down-regulated miRNAs were used to predict mRNAs based on online data mirTarBase. Subsequently, we intersected the predicted mRNAs with the DE mRNAs that was earlier analyzed. In this process, we should ensure: 1. mRNA and lncRNA are positively correlated 2. mRNA and lncRNA have a common related miRNA, and they are negatively correlated with miRNA expression, finally 14 dysregulated miRNAs and 10 dysregulated lncRNAs, as well as 16 dysregulated mRNAs, were found for ceRNA construction. Consequently, a ceRNA network was constructed based on the above data and visualized using Cytoscape v3. 7. 1 (Fig. 5) to provide insight into the mediation mechanisms of mRNA-miRNA combination.

3. 6. Prognosis of lncRNA in ceRNA network

To dig out the significant lncRNAs. We made prognosis analysis of those differential lncRNAs in ceRNA network. There were several lncRNAs associated with the prognosis of AML. For the up-regulated lncRNAs, there were two lncRNAs, ENSG00000235823 (OLMA -LINC, lincRNA) (Fig. 6A) and ENSG00000273001 (RP11-118K6.3, lincRNA) (Fig. 6B), related to AML survival. With the up-regulation of these two lncRNAs, the AML prognosis deteriorated, thus consistent with the poor prognosis of AML when CPT1B was highly expressed.

There were four lncRNAs associated with AML survival. The down-regulated lncRNA ENSG00000203804 (ADAMTSL4AS1, processed_transcript) (Fig. 6C) also named C1orf138. ADAMTSL4 is a member of ADAMTS-like gene family and encodes a protein with seven thrombospondin type 1 repeats. The thrombospondin type 1 repeat domain is present in proteins with diverse biological functions including cellular adhesion, angiogenesis and patterning of the developing nervous system. Besides, the down-regulated lncRNA ENSG00000237298 (TTN-AS1, antisense) (Fig. 6D) have vital roles in a variety of cancers as a tumor promoter. The other two

down-regulated lncRNAs, ENSG00000267009 (RP11-120M18.2, processed transcript) and ENSG00000269019 (AC005932.1, antisense), were not reported (Fig. 6F). Decreasing expressions in these four lncRNAs, simultaneously lead to a worse AML prognosis which was consistent with the poor prognosis of AML when CPT1B was highly expressed.

3. 7. Characteristics of CN-AML Patients with High and Low CPT1B Expression

CPT1B expressions were measured via quantitative PCR in BM samples from 325 CN-AML patients in order to verify the prognosis role of CPT1B in AML. The interquartile range of CPT1B transcript level ranged from 1.068 to 5.915. After to plot The survival curve (Fig. 7A) plotted using the quartile method enabled the election of a value at 25% as the cut-off for categorising patients into high and low CPT1B expressors. A value of 3.92 (2.37, 7.28) and 0. 67 (0.49, 0.89) were the median and interquartile range in high and low CPT1B expressors, respectively. Clinical characteristics of patients with high and low CPT1B expression are shown in Table 1. High expressors had an older age (55 vs 50, $P = 0.045$) than low expressors. High CPT1B expression patients had a higher PLT counts (50 vs 40.5, $P = 0.053$). However, there was no significant correlation between CPT1B expression and other variables including sex, white blood cell counts (WBC), hemoglobin levels, percentage of bone marrow blasts, genes of FLT3-ITD, CEBPA, NPM1, DNMT3A, IDH1 and IDH2 mutations (Table 1).

3. 8. Overexpression of CPT1B Associated with Poor Clinical Outcome in CN-AML Patients

In our 325 CN-AML patients, three years overall survival (OS) rate and event-free survival (EFS) rate were 45.5% and 38.9%, respectively. CPT1B in high expression group was significantly associated with short OS ($P = 0.026$) but there was no significant association between CPT1B expression and EFS ($P = 0.183$) (Fig. 7B & C). Thus, the expression of CPT1B was indeed a poor predictor in different populations. In addition, to confirm the prognostic function of CPT1B and exclude the potential confounders factors, multivariable analysis was performed in the entire CN-AML and multivariable models of OS was built after adjusting for the known risk factors such as age, WBC and genes including FLT3ITD, CEBPA mutations, etc. CPT1B expression was still an independent prognostic factor after adjusting ($P = 0.021$) (Table 2). For the whole CN-AML population, people with high CPT1B expression had a 1.526-fold higher OS risk ($P = 0.021$). Other negative factors included elder age (OS: $P < 0.001$), WBC count (OS: $P < 0.001$), wild type of NPM1 (OS: $P = 0.027$), the presence of FLT3-ITD (OS: $P < 0.001$), DNMT3A (OS: $P = 0.002$) and CEBPA (OS: $P < 0.001$)

4 Discussion:

In recent years, many studies have confirmed that fatty acid oxidation played an important role in tumorigenesis and development as well as determined the prognostic and therapeutic target role of fatty acid rate-limiting enzyme CPT1A. Elsewhere, studies on another subtype named CPT1B are limited. Moreover, the clinical and prognostic significance of CPT1B in AML, especially in CN-AML, is unknown. In this study, we showed that CN-AML patients with high CPT1B expression obtained higher age and hemoglobin levels. These results supported the hypothesis that increased CPT1B expression is associated with an increased risk of refractory and relapse in leukemia. Indeed, high CPT1B expressors had short overall survival times in two independent patient cohorts. General, CPT1B is an independent risk factor for poor prognosis of

AML.

Expression profiles of CPT1B were conducted to identify potential biological behavior of high CPT1B in CN-AML. First, we performed protein interaction network (PPI) analysis on 413 significantly up-regulated and 164 significantly down-regulated genes in high CPT1B expressors via STRING, respectively, before analyzing the hub genes of nodes in cystoscape. The KEGG pathways enrichment for the hub genes revealed many pathways which were likely to promote the development of acute myeloid leukemia [21, 22]. Besides, hub genes included in corresponding pathways can be used as potential drug targets in the future. Identification of hallmarks among the ten groups of CN-AML patients via GSEA clarified several upregulated pathways which showed why high CPT1B expression was associated with poor prognosis of CN-AML. Interestingly, the aberrant changes between CPT1B high and low expression groups were related to metabolisms. Fatty acid pathways especially fatty acid metabolism showed an up-regulation trend in CPT1B high expressors (Fig. 2C) which was previously reported being involved by CPT1B and promote tumor cell proliferation. Analysis of mRNA levels further confirmed the prognosis and metabolic function of CPT1B.

By further analyzing the differential miRNAs between the high and low CPT1B expressors, 82 up-regulated miRNAs and 48 down-regulated miRNAs were identified. From the KEGG enrichment analysis combination of the differential miRNAs with the pathways of differential mRNAs, several commonalities were established that pointed out to possible regulatory pathways of CPT1B. miRNAs were then integrated with its pathways to help us find several significant ones: miRNA-18a, which up-regulated in high CPT1B expressor, is highly expressed in many malignancies. The altered miR-18a potentially mediates a regulatory role in a variety of physiological and pathological processes such as cell proliferation, apoptosis, epithelial-mesenchymal transition (EMT), tumorigenesis, cancer invasion and metastasis [23]. Studies had shown that the up-regulated miRNA-182 was associated with poor tumor prognosis [24]. On the other hand, up-regulated miRNA-29a can cause abnormal development of bone marrow and trigger acute myeloid leukemia through abnormal self-renewal ability of hematopoietic progenitor cells [25]. From the properties of miRNAs that were positive correlation with high CPT1B expression, verified the prognostic function of CPT1B from the other side. Furthermore, fatty acid pathways such as fatty acid metabolism, fatty acid elongation, citrate cycle (TCA cycle) revealed a significant change. With these results, it is possible to further reveal the correlation between CPT1B and AML prognosis as well as the metabolic characteristics of CPT1B.

Developing miRNA-mRNA network facilitates understanding the regulatory mechanism between differential miRNAs and mRNAs. The identified miRNA-509 which was upregulated in the high CPT1B expressor was related to the decrease in tumor progression [26]. This indicated the potential of miRNA-509 to mediate cell progress as well as being a progress promoter in AML, which differs with other cancers since AML is aerobic cancer while others are hypoxic cancers. miRNA-339 promotes the development of Stem Cell Leukemia/Lymphoma syndrome via downregulation of the BCL2L1 and BAX pro-apoptotic genes [27]. These results supported the poor prognosis of CPT1B from the related miRNA level. Following the identification of several mRNAs participating in hematopoietic progenitor cell differentiation and metabolic process after function analysis, it is evident that CPT1B high expressor was associated with poor prognosis as well as verifying CPT1B as a metabolic molecule.

The hypothesis of lncRNA playing a significant role in the deterioration of AML prognosis due to high expression of CPT1B was tested by analysing the differential lncRNAs and ascertain with previous studies on CPT1B expressors. Two lncRNAs were up-regulated, ENSG00000235823 (OLMALINC, lincRNA) and ENSG00000273001 (RP11-118K6.3, lincRNA). Notably, ENSG00000235823 (OLMALINC, lincRNA) is a long intervening noncoding RNA (lincRNA) present in human liver co-expression network (n = 75 genes) and strongly associated with statin use and serum triglycerides (TGs). The expression of OLMALINC in the liver was highly correlated with the expression of known cholesterol biosynthesis genes and stearoyl-coenzyme A desaturase (SCD) [28]. As for ENSG00000273001 (RP11-118K6.3, lincRNA), though it has not been reported, there have been many papers revealing the function of other lncRNAs in RP11 family. For instance, RP11-108K3.2 has recently been identified as a novel long non-coding RNA (lncRNA) transcript following its significant association with the risk of colorectal cancer [29]. The down-regulated lncRNAs were ENSG00000203804 (ADAMTSL4-AS1, processed_transcript), ENSG00000237298 (TTN-AS1, antisense), ENSG00000267009 (RP11-120M18.2, processed_transcript) and ENSG00000269019 (AC005932.1, antisense). Though ADAMTSL4-AS1 and AC005932.1 have not been reported, hence their further studies are recommended. However, TTN-AS1 plays a significant function in a variety of cancers as a tumor promoter. For example, it facilitates proliferation, invasion and epithelial-mesenchymal transition of breast cancer cells by regulating miR - 139 - 5p/ZEB1 axis [30]. LncRNA TTN-AS1 was reported to communicate with a variety of miRNAs to function in various cancers but not identified in AML. RP11-120M18.2 was also involved in the RP11 family that functions in cancer progression and metabolism. The relationship between the expression changes of lncRNAs and the prognosis of AML proved CPT1B as a risk factor in AML as well as providing insights for our follow-up research.

In summary, the present study demonstrated that high CPT1B expression potentially predicts adverse survival for patients with CN-AML in two independent cohorts. A series of differential mRNAs, miRNAs and lncRNAs between the high and low CPT1B expressors were obtained via bioinformatics analysis. Besides, the enrichment analysis of differential mRNAs and miRNAs provides several common pathways like P53, mTOR and so on which were likely to participate in the regulation of CPT1B in AML. The construction of the miRNA-mRNA network revealed a series of important regulatory relationships. Consequently, we identified 2 down-regulated miRNAs and a set of targeted genes co-expressed with CPT1B. These biological links can explain the poor prognosis of CPT1B. On the other hand, the luciferase reporting experiment needs to be further developed in the future. Consequently, the ceRNA network constructed by combining differential mRNAs, miRNAs and lncRNAs provides insights to research perspectives and directions. Therefore, this article revealed the potential of CPT1B as an independent risk factor for poor prognosis as well as a metabolic molecule in CN-AML from the mRNA, miRNA and lncRNA level. Moreover, by analyzing the entire possible regulatory network, it further provides ideas for subsequent mechanism research.

5 Conclusion:

In this study, we report the role of CPT1B as an independent prognostic marker for AML. In addition, the findings of this study reveal the possible regulatory network of CPT1B in regulation of AML survival. In summary, the findings of this study provide information on the function and

mechanisms of action of CPT1B in AML.

Ethics approval and consent to participate

The study was approved by the Institutional Review Boards of the First Affiliated Hospital of Zhejiang University.

Consent for publication

None of the individual person's data is in this text for publication.

Availability of data and materials

In this study, 68 CN-AML patients with survival information were obtained from TCGA (<https://tcga-data.nci.nih.gov/tcga/>) .

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qing Ling and Jie Jin: Research design, data methodology, data interpretation, final manuscript writing and approval. Shihui Mao: literature search, data Integration, molecular genetic studies and approval of the final manuscript. Jiajia Pan, Wenwen Wei, Yu Qian, Fenglin Li, Shujuan Huang, Wenle Ye, Xiangjie Lin, Jiansong Huang, Jinghan Wang: data verification, final manuscript discussion and approval.

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Figures

Fig.1

Figure 1

Survival curves of AML patients.a High CPT1B expression is associated with poor AML OS(Analysis by online database GEPIA).b CPT1B expression in AML patient is significantly higher than it in normal people(red presents AML patient while grey presents normal people).

Fig.2

Figure 2

Associations Between Genome-wide Expression Profiles and CPT1B Expression.a Top30 hub genes in up-regulated differential genes.b Top30 hub genes in down-regulated differential genes.c.Differential hallmarks between high and low expression of CPT1B.d Top30 KEGG pathways about up-regulated hub genes.e Top30 KEGG pathways about down-regulated hub genes.

Fig.3

Figure 3

KEGG analysis of dysregulated miRNAs.a KEGG analysis of up-regulated miRNAs.b KEGG analysis of down-regulated miRNAs.

Fig.4

Figure 4

Fig.6

Figure 6

Prognosis of dysregulated lncRNAs. a Upregulated of ENSG00000235823 predicts poor outcome. b Upregulated of ENSG00000273001 predicts poor outcome. c Downregulated of ENSG00000203804 predicts poor outcome. d Downregulated of ENSG00000237298 predicts poor outcome. e Downregulated of ENSG00000267009 predicts poor outcome. f Downregulated of ENSG00000269019 predicts poor outcome

Fig.7

Figure 7

Survival curves of CN-AML patients.a Using the quartile method to plot the survival curve of CPT1B in AML. b High CPT1B expression is associated with poor AML OS. c High CPT1B expression is related to poor AML EFS.

Supplementary Files

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