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Research Paper

High Expression of *CPT1A* Predicts Adverse Outcomes: A Potential Therapeutic Target for Acute Myeloid Leukemia

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ABSTRACT

Carnitine palmitoyl transferase 1A (*CPT1A*) protein catalyzes the rate-limiting step of *Fatty-acid oxidation* (*FAO*) pathway, which can promote cell proliferation and suppress apoptosis. Targeting *CPT1A* has shown remarkable anti-leukemia activity. But, its prognostic value remains unclear in Acute Myeloid Leukemia (AML). In two independent cohorts of cytogenetically normal AML (CN-AML) patients, compared to low expression of *CPT1A* ($CPT1A^{low}$), high expression of *CPT1A* ($CPT1A^{high}$) was significantly associated with adverse outcomes, which was also shown in European Leukemia Network (ELN) Intermediate-I category. Multivariable analyses adjusting for known factors confirmed $CPT1A^{high}$ as a high risk factor. Significant associations between $CPT1A^{high}$ and adverse outcomes were further validated whether for all AML patients (OS: $P = 0.008$; EFS: $P = 0.002$, $n = 334$, no M3) or for National Comprehensive Cancer Network (NCCN) Intermediate-Risk subgroup (OS: $P = 0.021$, EFS: $P = 0.024$, $n = 173$). Multiple omics analysis revealed aberrant alterations of genomics and epigenetics were significantly associated with *CPT1A* expression, including up- and down-regulation of oncogenes and tumor suppressor, activation and inhibition of leukemic (AML, CML) and immune activation pathways, hypermethylation enrichments on CpG island and gene promoter regions. Combined with the previously reported anti-leukemia activity of *CPT1A*'s inhibitor, our results proved *CPT1A* as a potential prognosticator and therapeutic target for AML.

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1. Introduction

Acute myeloid leukemia (AML) represents a group of myeloid malignancies with remarkably heterogeneous outcomes. Finding effective prognostic biomarkers has been being one of the most urgent clinical needs and research hotspots. Cytogenetically normal acute myeloid leukemia (CN-AML) accounts for about one-half of total AML and constitutes the main body of intermediate-risk AML (Dohner et al., 2010). Since there are no microscopically detectable chromosome abnormalities in leukemic blasts, CN-AML shows a particular uniformity of cytogenetics and provides a perfect platform of biomarker recognition for AML. Many kinds of molecular signals (such as DNA mutations, aberrant expression of mRNA and microRNAs) have been identified independently as favorable or adverse prognostic biomarkers. Recent studies showed that metabolic signatures were involved in leukemogenesis and could inspire novel therapeutic regimens for AML (Cheong et al., 2012).

Aggressive metabolic changes are key hallmarks of cancer (Hanahan and Weinberg, 2000), Warburg effect of aerobic glycolysis has been regarded as an important bioenergetic source for rapid cell proliferation (Hsu and Sabatini, 2008). Beyond the Warburg effect, other effects, especially fatty acid oxidation (*FAO*) (Carracedo et al., 2013), also show important roles in the cancer pathogenesis. Series reports of therapeutic applications for metabolic signatures have appeared for AML, including a distinct glucose metabolism signature proved as a prognostic biomarker (Chen et al., 2014), glutamine uptake (Willems et al., 2013) and arginine dependence (Mussai et al., 2015) reported as novel therapeutic targets. Particular concerns were paid on *FAO*, an original article and editorial discussed function blocking of *FAO* with *CPT1A*'s inhibitor (ST1326) and its inherent mechanisms for anti-leukemia treatment

Abbreviations: AML, Acute myeloid leukemia; CN-AML, cytogenetically normal AML; *CPT1A*, Carnitine palmitoyl transferase 1A; $CPT1A^{high}$, high expression of *CPT1A*; $CPT1A^{low}$, low expression of *CPT1A*; *FAO*, fatty-acid oxidation; OS, overall survival; EFS, event-free survival; ELN, European Leukemia Net; NCCN, National Comprehensive Cancer Network.

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(Ricciardi et al., 2015, Samudio and Konopleva, 2015). But, up to now, the prognostic value of *CPT1A* expression remains unclear for AML patients.

Here, we presented *CPT1A*^{high} as an adverse prognostic biomarker for AML with concrete data, and also explored the distinctive genomic and epigenomic patterns associated with *CPT1A* expression. Compared to the existing basic experiments of *CPT1A*, our work offered more direct evidences for using *CPT1A* expression as a prognostic biomarker in risk stratification or as a potential therapeutic target for AML patients.

2. Materials & Methods

2.1. Patients and Treatment

The first cohort was derived from a whole AML cohort ($n = 334$, no M3), which included 156 *de novo* CN-AML patients (median age, 50 years, range: 16–77 years), which were all diagnosed and collected at Erasmus University Medical Center (Rotterdam) between 1990 and 2008. 83% of patients ($n = 129$) were aged under 60 years (younger patients) and 17% patients were ≥60 years (older patients). The study was approved by the institutional review boards at Weill Cornell Medical College and Erasmus University Center, and all subjects provided written informed consent in accordance with the Declaration of Helsinki. The methods were carried out in accordance with the approved guidelines. All patient were uniformly treated under the study protocols of Dutch-Belgian Cooperative Trial Group for Hematology Oncology (HOVON, details of therapeutic protocol available at http://www.hovon.nl). All clinical, cytogenetic and molecular information as well as microarray data of these patients can be publicly downloaded at the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo, *GSE6891* (Verhaak et al., 2009)). All samples contained 80–100% blast cells after thawing. Conventional cytogenetic examination of at least 20 metaphases from bone marrow (BM) was used to determine normal karyotype. RT-PCR assays were used to assess the mutations of *NPM1*, *CEBPA*, *N-RAS*, *K-RAS*, *IDH1* and *IDH2*, the presence of *FLT3*-ITD and *FLT3*-TKD [D835].

Another independent cohort included 162 previously untreated CN-AML patients (median age: 57.5, range: 17–83 years), which also received uniform intensive double-induction and consolidation chemotherapy provided by the multicenter AMLCG-1999 trial of German AML Cooperative Group between 1999 and 2003. The AMLCG-1999 clinical trial was approved by the local institutional review boards, and written informed consent for each patient were obtained in accordance with the Declaration of Helsinki. The methods were carried out in accordance with the approved guidelines. Microarray data as well as patient information were also publicly available (GEO accession number: *GSE12417* (Metzeler et al., 2008)).

2.2. Microarray and Sequencing Data Analyses

Several previously published dataset were used for gene expression profiles, including *GSE6891* (Verhaak et al., 2009), *GSE12417* (Metzeler et al., 2008), *GSE1159* (Valk et al., 2004), *GSE9476* (Stirewalt et al., 2008) and *GSE30029* (de Jonge et al., 2011), all of which can be obtained from GEO. Microarray expression profiles were obtained by Affymetrix Human Genome 133 plus 2.0 and U133A Gene Chips. All experiments' design, quality control and data normalization were in line with the standard Affymetrix protocols. Expression profiles of mRNA and microRNA were obtained by high throughput sequencing (RNA-Seq), and genome-wide methylation data were obtained by Illumina infinium 450K beadchips, derived from the Cancer Genome Atlas (TCGA), which provided 73 CN-AML patients with all mRNA and microRNA expression and methylation profiles (Cancer Genome Atlas Research, 2013). For microarray data, expression levels of a gene were computed as the mean value of all probe sets annotated to it, while for RNA-Seq data, expression levels of mRNA and microRNA were computed as RPKM and RPM (**R**eads **P**er **K**ilo-base per **M**illion reads). To choose the appropriate cut-off value for subdivision, we accessed the distribution of *CPT1A* expression level and compared survivals for the 156 CN-AML patients dividing into 4 quartiles, results represented a normal distribution (Fig. S1A) and evident distinction along the median value (Fig. S1B and C). Thus, median value of *CPT1A* expression was used to divide patients into *CPT1A*^{high} and *CPT1A*^{low} groups. Other dividing of patients according to a gene's expression (such as *ERG*, *WT1* and *DMNT3A etc.*) were dealt using the same strategy.

2.3. Statistical Analyses

The definition of overall survival (OS) was the time from date of diagnosis to death due to any causes. Event free survival (EFS) was defined as the time from date of diagnosis to removal from the study due to the absence of complete remission (CR), relapse or death. The Kaplan-Meier method and log-rank test were used to estimate the association between *CPT1A* expression and OS, EFS. The Fisher exact and Wilcoxon rank-sum tests were used, respectively for categorical and continuous variables, to assess the association between expression levels and clinical, molecular characteristics. Multivariable hazards models were used to evaluate the impacts of *CPT1A* expression to OS and EFS in the presence of other known risk factors. Student's *t*-test and multiple hypothesis correction (False Discovery Rate, FDR) were used to identify differences in genome-wide gene, microRNA and methylation profiles between *CPT1A*^{high} and *CPT1A*^{low} groups. All analyses were performed using the R 3.2.3 software packages.

3. Results

3.1. Overexpression of CPT1A in AML Patients

Three public microarray datasets were used to compare *CPT1A* expression between AML patients and heathy donors using bone marrow (BM), peripheral blood (PB) and CD34+ cells. For the comparison of BM samples, *CPT1A* was highly expressed in AML (Fig. 1A, $P = 0.049$, 7 AML *vs* 10 NBM, ***GSE9476***), which was validated in PB samples (Fig. 1B, $P < 0.001$, 19 AML *vs* 10 normal, ***GSE9476***). Additionally, CD34+ cells derived from AML patients and healthy donors were used to further verify *CPT1A*'s overexpression in AML patients (Fig. 1C, $P < 0.001$, 46 AML CD34+ *vs* 31 normal CD34+, ***GSE30029***). Besides, higher expression levels of *CPT1A* was shown in various different AML-subtypes than normal BM, including 19 CBFB-MYH11, 115 CN-AML, 10 Complex, 17 *MLL*-translocation, 86 Others, 22 *RUXN1-RUNX1T1* and 5 normal BM (Fig. S2, ***GSE1159***). All these results showed significant overexpression of *CPT1A* in AML patients.

3.2. Different Molecular Characteristics Between CPT1A^{high} and CPT1A^{low} Groups

In the cohort of 156 CN-AML derived from ***GSE6891*** (334 AML, no M3), patients in *CPT1A*^{high} group tended to be younger ($P = 0.03$) and belong more to FAB M1 ($P = 0.03$) than that in *CPT1A*^{low}. *CPT1A*^{high} group carried more *FLT3*-ITD than *CPT1A*^{low} ($P = 0.05$), while no additional significant associations between *CPT1A* expression and other mutations were found. In addition, *CPT1A*^{high} was more likely to accompany with higher expression of many known adverse prognostic biomarkers, such as *ERG* ($P < 0.001$), *BAALC* ($P < 0.04$), *WT1* ($P < 0.001$), *DNMT3A* ($P < 0.001$), *DNMT3B* ($P = 0.006$), *MAPKBP1* ($P = 0.04$), *ITPR2* ($P < 0.001$), *ATP1B1* ($P < 0.001$), *RUNX1* ($P < 0.001$), *TCF4* ($P = 0.002$) and *CXXC5* ($P < 0.001$). (See Table 1 and Fig. S3A)

3.3. CPT1A^{high} Is Associated With Adverse Outcomes in AML

Survival analyses were carried out in the whole cohort of 156 CN-AML patients and European Leukemia Net (ELN) Intermediate-I

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