Identification and validation of obesity-related gene *LEP* methylation as a prognostic indicator in patients with acute myeloid leukemia

Ting-juan Zhang\(^{1,2,3}\), Zi-jun Xu\(^{2,3,4}\), Yu Gu\(^{1,2,3}\), Ji-chun Ma\(^{2,3,4}\), Xiang-mei Wen\(^{2,3,4}\), Wei Zhang\(^{1,2,3}\), Zhao-qun Deng\(^{2,3,4}\), Jun Qian\(^{1,2,3,*}\), Jiang Lin\(^{1,2,3,4,*}\) and Jing-dong Zhou\(^{1,2,3,*}\)

**Abstract**

**Background:** Obesity confers enhanced risk for multiple diseases including cancer. The DNA methylation alterations in obesity-related genes have been implicated in several human solid tumors. However, the underlying role and clinical implication of DNA methylation of obesity-related genes in acute myeloid leukemia (AML) has yet to be elucidated.

**Results:** In the discovery stage, we identified that DNA methylation-associated *LEP* expression was correlated with prognosis among obesity-related genes from the databases of The Cancer Genome Atlas. In the validation stage, we verified that *LEP* hypermethylation was a frequent event in AML by both targeted bisulfite sequencing and real-time quantitative methylation-specific PCR. Moreover, *LEP* hypermethylation, correlated with reduced *LEP* expression, was found to be associated with higher bone marrow blasts, lower platelets, and lower complete remission (CR) rate in AML. Importantly, survival analysis showed that *LEP* hypermethylation was significantly associated with shorter overall survival (OS) in AML. Moreover, multivariate analysis disclosed that *LEP* hypermethylation was an independent risk factor affecting CR and OS among non-M3 AML. By clinical and bioinformatics analysis, *LEP* may be also regulated by miR-517a/b expression in AML.

**Conclusions:** Our findings indicated that the obesity-related gene *LEP* methylation is associated with *LEP* inactivation, and acts as an independent prognostic predictor in AML.

**Keywords:** Obesity, *LEP*, Methylation, Prognosis, AML

© The Author(s) 2021. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

**Background**

Acute myeloid leukemia (AML) is an aggressive hematological malignancy characterized by clonal proliferation of the hematopoietic progenitor cells [1]. Clinical outcome of AML is heterogeneous due to the cytogenetically and molecularly diverse [1]. Despite the improved treatment regimens, more than 50% of AML patients experience short-term recurrence [1]. Early identification of patients with poor prognosis, and then given intervene accordingly can help to improve AML survival. Currently,
the 2017 European LeukemiaNet (ELN) risk stratification by genetics is widely accepted, but there is a practical limitation to the definition of genetic risk, especially in patients falling in the intermediate group [2]. Therefore, additional prognostic factors are needed.

Obesity has been verified as an independent health risk and is significantly correlated with the development of metabolic disorders, including hyperlipidemia, type 2 diabetes mellitus, hypertension, stroke, and cardiovascular disease. Furthermore, strong evidences have proved the links between body mass index (BMI) and various cancers including the most forms of tumor-based cancer and hematological malignancies [3–6]. A number of studies have showed the increased risk of cancer including leukemia incidence in obese patients [7, 8], and excess fat mass is associated with both enhanced incidence and lower survival for pediatric leukemia [9]. Moreover, obesity independently conferred poor prognosis in AML [10]. Yan et al. [11] revealed that Fatty acid-binding protein 4 mechanistically linked obesity with aggressive AML by enhancing aberrant DNA methylation. To date, various genes such as LEP, LEPR, NPY, ADIPOQ, FTO, MC4R, PCSK1, and POMC are implicated and have a direct role in obesity [12].

To the best of our knowledge, epigenetic alterations have been suggested as a molecular mechanism mediating gene expression, and also described as a potential early cancer-related biomarker with strategies for diagnostic, prognostic or cancer screening procedures being developed [13, 14]. To date, epigenetic mechanisms including DNA methylation and aberrant microRNAs (miRNAs) expression involving in obesity-related genes have been reported especially in human cancers with prognostic significance [12, 15]. However, the underlying role and clinical implication of DNA methylation of obesity-related genes in AML has yet to be elucidated.

Materials and methods

Patients and samples

The first cohort of 200 AML patients from The Cancer Genome Atlas (TCGA) databases included in this study was used in the discovery stage for the identification of prognostic methylation-related genes [16]. Among the cohort, there are 173 cases with expression data whereas 194 patients with methylation data [17, 18]. In addition, DiseaseMeth version 2.0 (http://bio-bigdata.hrbmu.edu.cn/diseasemeth/analyze.html) was applied to compare the methylation difference between these AML patients with controls.

A second cohort of 25 healthy donors and 111 de novo AML patients treated at the Affiliated People’s Hospital of Jiangsu University were also enrolled, and used in the validation stage for targeted bisulfite sequencing. In addition, expanded samples of the sequencing cohort, including 172 AML patients (161 de novo AML and 11 MDS-derived AML) and 46 healthy donors, were used in the validation stage for real-time quantitative methylation-specific PCR (qMSP). AML patients were diagnosed and classified according to the 2016 World Health Organization (WHO) criteria [19]. Treatment regimens for AML patients were as reported [20, 21]. The detection of gene mutations in this study was described as our previous reports [20, 21]. After informed consents were obtained from all participants, bone marrow (BM) was collected at diagnosed time, and were separated to obtain BM mononuclear cells (BMMNCs) by density-gradient centrifugation using Lymphocyte Separation Medium (Solarbio, Beijing, China) [20, 21]. The current study was approved by the Ethics Committee of Affiliated People’s Hospital of Jiangsu University.

Targeted bisulfite sequencing

The target gene methylation was detected by Targeted bisulfite sequencing—MethylTarget, which was performed in Genesky Biotechnologies Inc. (Shanghai, China) as our previous investigations [22, 23]. The primers used for LEP were shown in Additional file 1: Table S1.

Reverse transcription and qPCR

Reverse transcription was carried out as our previous studies [20, 21]. The detection of LEP mRNA expression was performed by real-time quantitative PCR (qPCR) using AceQ qPCR SYBR Green Master Mix (Vazyme, Piscataway, NJ). The reference gene ABL1 mRNA, examined by 2 × SYBR Green PCR Mix (Multisciences, Hangzhou, China), was detected to calculate the abundance of LEP mRNA expression. The qPCR primers used for LEP expression detection were listed in Additional file 1: Table S1. Relative LEP mRNA expression was calculated using 2−ΔΔCT method.

Bisulfite modification and qMSP

Genomic DNA was bisulfite converted as our previous reports [21, 22]. The detection of LEP methylation level was evaluated by qMSP with primers shown in Additional file 1: Table S1. The reference gene ALU methylation level was also detected. Relative LEP methylation level was calculated using 2−ΔΔCT method.

Bioinformatics analysis and bioinformatics prediction of miRNA targets

Differential expression analysis for RNA/miRNA sequencing data was calculated using the raw read counts with the R/Bioconductor package “edgeR”, all analyses were controlled for the false discovery rate (FDR) by
Statistical analyses
SPSS 20.0 and GraphPad Prism 5.0 were conducted to perform statistical analyses. Mann–Whitney’s U/Kruskal–Wallis followed by Dunn’s post hoc test and Pearson’s $\chi^2$/Fisher’s exact test were used for the comparison of continuous and categorical variables, respectively. Correlation analysis between LEP methylation and methylation/expression was performed by Spearman test. The Receiver operating characteristic (ROC) curve and area under the ROC curve (AUC) were used to evaluate LEP methylation level in distinguishing AML from controls. Complete remission (CR) was evaluated after 1–2 course of chemotherapy. Overall survival (OS) and leukemia free survival (LFS) were defined as previous report [20]. Survival analysis regarding the effect of LEP methylation on OS and LFS was analyzed by Kaplan–Meier analysis and Cox regression analysis (univariate and multivariable). A two-sided $P$ less than 0.05 was seen as statistically significant.

Results
Identification of prognostically obesity-related genes correlated with DNA methylation in AML
We first used TCGA data to identify the prognostically obesity-related genes including LEP, LEPR, NPY, ADIPOQ, FTO, MC4R, PCSK1, and POMC in AML. Prognostic value of these genes was analyzed in two groups divided by the median expression level of each gene respectively. In total AML and cytogenetically normal AML (CN-AML) patients, Kaplan–Meier analysis showed that only LEP expression was positively associated with OS ($P=0.013$ and 0.007, Fig. 1a) and LFS ($P=0.025$ and 0.062, Additional file 2: Figure S1), suggesting the prognostic effect of LEP expression in AML.

DNA methylation plays a crucial role in regulating gene expression. We next investigated the association between these obesity-related gene expression and methylation in AML. Among the eight genes, methylation data was available for LEP, LEPR, FTO, PCSK1, and POMC. Significantly negative association was shown in LEP ($R=-0.176$, $P=0.021$), LEPR ($R=-0.379$, $P<0.001$), FTO ($R=-0.361$, $P<0.001$), PCSK1 ($R=-0.229$, $P=0.003$), and POMC ($R=-0.744$, $P<0.001$) genes (Fig. 1b). These data suggested LEP, LEPR, FTO, PCSK1, and POMC genes methylation may play main roles in regulating gene expression during leukemogenesis, while LEP showed a very weak association. Moreover, we further identified that LEP promoter CpG island was hypermethylated in AML by using the DiseaseMeth version 2.0 ($P<0.001$, Fig. 1c).

Abnormal LEP promoter methylation in AML by targeted bisulfite sequencing
To validate the methylation pattern of LEP in AML, we analyzed CpG island methylation located at the LEP promoter region (Fig. 2a) by targeted bisulfite sequencing in BMMNCs samples of 25 controls and 111 de novo AML patients. The sequencing mean bait coverage attached 1694×, and Q30 was 75.56% [22, 23]. The targeted sequencing results exhibited that the level of LEP methylation in AML patients was markedly higher than that in controls ($P<0.001$, Fig. 2b).

Further confirmation of LEP methylation in a larger cohort of AML by qMSP
In order to explore whether LEP methylation could be helpful utilized in patients diagnosis, prognosis and risk/treatment assessment, we further expanded the patients samples including 46 controls, 161 primary AML and 11 secondary AML to explore clinical implication of LEP methylation by using a more rapid and convenient methodology—qMSP. The primers for qMSP were designed located inside the sequencing primer (Fig. 2c), and the results analyzed by qMSP results was positively associated with the results by targeted bisulfite sequencing ($R=0.404$, $P<0.001$, Fig. 2c). In addition, LEP promoter hypermethylation in primary and secondary AML was further confirmed by qMSP (both $P<0.001$, Fig. 2d). However, LEP methylation showed no significant
difference between primary and secondary AML (P=0.680, Fig. 2d). We next detected LEP expression in controls and AML patients with available RNA samples by qPCR. LEP expression was significantly decreased in AML (P<0.001, Fig. 2e), and was inversely correlated with LEP methylation (R=-0.338, P=0.009, n=59, Spearman test).

Clinical implication of LEP methylation in AML
ROC curve analysis exhibited that LEP promoter methylation may be severed as an underlying biological marker for distinguishing AML from controls with an AUC of 0.803 (95% CI 0.747–0.858, P<0.001, Fig. 2f). By the ROC analysis, LEP methylation at the value of 1.011 was set as cutoff point due to the sensitivity was 60.5% and the specificity was 100%. According to the set point, we divided AML patients into two groups to analyze the specificity was 100%. According to the set point, we divided AML patients into two groups to analyze the clinical significance of LEP methylation. No significant differences were found between two groups with regard to age, white blood cells, and hemoglobin (P>0.05, Table 1). However, LEP hypermethylation tended to be associated with male patients and higher BM blasts (P=0.057 and 0.064, respectively, Table 1), and significantly correlated with lower platelets (P=0.046, Table 1). Moreover, there were significant differences between two groups in the distribution of French-American-British (FAB) classifications and karyotypes (P=0.044 and 0.042, respectively, Table 1). LEP hypermethylation was less frequently occurred in M3/t(15;17) subtypes (P=0.003 and 0.001, respectively). Moreover, there were no significant associations between LEP hypermethylation and gene mutations besides N/R-RAS mutations with a trend (P=0.098, Table 1).

LEP methylation was associated with prognosis in AML
Firstly, we revealed the significant association of LEP methylation with CR rate in AML patients. Notably, CR rate in LEP hypermethylated patients was significantly lower than that in LEP non-hypermethylated patients among whole-cohort AML and non-M3 AML (P=0.011 and 0.049, respectively, Table 1). In CN-AML, we did not observe the significant difference for CR between LEP hypermethylated and non-hypermethylated patients (P=0.105, Table 1). Since the significant associations of LEP methylation with CR were observed among whole-cohort AML and non-M3 AML, Logistic regression analysis was performed to confirm the effect of LEP methylation on CR. After adjusting for the well-known prognostic factors, LEP hypermethylation acted as an independent risk factor negatively affecting CR in both whole-cohort AML and non-M3 AML patients (P=0.017 and 0.015, respectively, Tables 2 and 3).

Secondly, we also analyzed the effect of LEP methylation on OS and LFS in AML patients. Kaplan–Meier analysis indicated that LEP hypermethylated patients exhibited shorter OS time than LEP non-hypermethylated patients among total AML, non-M3 AML and CN-AML patients (P=0.010, 0.050, and 0.028, respectively, Fig. 3a, c, e). For LFS, significant difference was only observed in total AML between two groups (P=0.030, 0.081, and 0.057, respectively, Fig. 3b, d, f). Furthermore, by Cox regression analysis, LEP hypermethylation could severe as a prognostic biomarker independently affecting OS among total AML with a trend (P=0.052, Table 4) and non-M3 AML patients (P=0.041, Table 5).

MiRNA signatures correlated with LEP in AML
Due to a very weak correlation of LEP expression with LEP methylation in AML patients from both TCGA cohort and validation data, we thought that LEP expression in AML was not only regulated by LEP methylation, and other mechanism also involved such as miRNAs. To gain insights into the molecular signatures associated with LEP in AML, we first compared the transcriptomes of miRNAs expression signatures in lower and higher LEP expression groups (based on the median level of LEP expression) of AML patients from TCGA datasets. A total of 83 differentially expressed miRNAs (included 71 positively correlated and 12 negatively correlated) (FDR<0.05, P<0.05, |log2 FC|>1; Fig. 4a; Additional file 3) were identified between two groups. The negatively correlated miRNAs such as miR-10a was identified to be significantly associated with AML with NPM1 mutation [27], whereas the other genes including miR-582,
miR-517, miR-511, miR-508, miR-518c, miR-520g, and miR-187 were less investigated. Moreover, LEP was identified as a direct target of 69 miRNAs by bioinformatics prediction (Fig. 4b, Additional file 4). Of these miRNAs, miR-517a/b was shared in both clinical data and

Table 1 Comparison of clinical and laboratory features between LEP hypermethylated and non-hypermethylated AML patients

| Patient’s features | Non-hypermethylated (n = 68) | Hypermethylated (n = 104) | P value |
|--------------------|------------------------------|--------------------------|---------|
| Sex, male/female   | 35/33                        | 69/35                    | 0.057   |
| Median age, years (range) | 55.5 (18–85)           | 55 (18–86)               | 0.872   |
| Median WBC, × 10⁹/L (range) | 12.5 (0.9–528.0)    | 18.4 (0.3–232.1)         | 0.626   |
| Median hemoglobin, g/L (range) | 76 (32–147)           | 79 (32–144)              | 0.951   |
| Median platelets, × 10⁹/L (range) | 50 (6–447)          | 38.3 (3–415)             | 0.046   |
| Median BM blasts, % (range) | 45 (5.5–97.5)        | 56.5 (1–99)              | 0.064   |
| FAB classifications |                              |                          | 0.044   |
| M0                 | 0                            | 2                        |         |
| M1                 | 5                            | 6                        |         |
| M2                 | 21                           | 45                       |         |
| M3                 | 19                           | 10                       |         |
| M4                 | 15                           | 20                       |         |
| M5                 | 7                            | 14                       |         |
| M6                 | 1                            | 5                        |         |
| No data            | 0                            | 2                        |         |
| Karyotypes         |                              |                          | 0.042   |
| Normal             | 24                           | 52                       |         |
| t(8;21)            | 4                            | 8                        |         |
| inv(16)            | 0                            | 2                        |         |
| t(15;17)           | 19                           | 8                        |         |
| +8                 | 2                            | 3                        |         |
| -5/-5q             | 1                            | 0                        |         |
| -7/7q              | 0                            | 1                        |         |
| t(9;22)            | 1                            | 1                        |         |
| 11q23              | 0                            | 2                        |         |
| Complex            | 7                            | 10                       |         |
| Others             | 7                            | 9                        |         |
| No data            | 3                            | 8                        |         |
| Gene mutations     |                              |                          |         |
| CEBPA (±)          | 4/53                         | 12/69                    | 0.187   |
| NPM1 (±)           | 5/52                         | 10/71                    | 0.587   |
| FLT3-ITD (±)       | 4/53                         | 8/73                     | 0.761   |
| C-KIT (±)          | 5/52                         | 3/78                     | 0.274   |
| N/K-RAS (±)        | 3/54                         | 12/69                    | 0.098   |
| IDH1/2 (±)         | 3/54                         | 2/79                     | 0.404   |
| DNMT3A (±)         | 2/55                         | 6/75                     | 0.470   |
| U2AF1 (±)          | 0/57                         | 3/78                     | 0.267   |
| SRSF2 (±)          | 3/54                         | 1/80                     | 0.306   |
| SETBP1 (±)         | 0/57                         | 2/79                     | 0.512   |
| CR, total AML (±)  | 33/26                        | 29/56                    | 0.011   |
| CR, non-M3 AML (±) | 20/22                        | 23/56                    | 0.049   |
| CR, CN-AML (±)     | 11/9                         | 14/29                    | 0.105   |

Patients’ blasts less than 20% with t(15;17) cytogenetic aberrations

WBC white blood cells, BM bone marrow, FAB French-American-British classification, CR complete remission
bioinformatics prediction, suggesting that \textit{LEP} may be also regulated by \textit{miR-517a/b} expression in AML (Fig. 4c).

**Discussion**

Obesity confers enhanced risk for multiple diseases including cancer, and is increasingly recognized as a growing cause of preventable cancer risk [3–6]. The DNA methylation alterations in obesity-related genes have been implicated in several human solid tumors [12, 15]. Previously, promoter methylation of obesity-related genes including \textit{LEP, NPY}, and \textit{LEPR} was involved in tumorigenesis of renal cell carcinoma, and \textit{LEPR} methylation was associated with prognosis, and predicted renal cell carcinoma recurrence [12, 28]. Herein, we for the first time evaluated prognostic value of obesity-related gene expression and methylation in AML. By the identification and validation stage, we finally revealed that \textit{LEP} methylation, negatively associated with \textit{LEP} expression, was independently associated with clinical outcome in AML.

The expression pattern and direct role of \textit{LEP} remains controversial in AML. Functional studies have showed
Fig. 3 Prognostic value of LEP methylation in AML patients. 

- **a, c, e** The impact of LEP methylation on overall survival among whole-cohort AML, non-M3-AML, and CN-AML patients, respectively.
- **b, d, f** The impact of LEP methylation on leukemia-free survival among whole-cohort AML, non-M3-AML, and CN-AML patients, respectively.

### Table 4 Cox regression analyses of variables for overall survival in AML patients

| Variables                        | Univariate analysis | Multivariate analysis |
|----------------------------------|---------------------|-----------------------|
|                                  | Hazard ratio (95% CI) | P value  | Hazard ratio (95% CI) | P value |
| LEP methylation                 | 1.639 (1.104–2.435)  | 0.014    | 1.515 (0.996–2.304)  | 0.052   |
| Age                             | 2.690 (1.839–3.933)  | 0.000    | 2.033 (1.364–3.031)  | 0.000   |
| WBC                             | 2.358 (1.613–3.447)  | 0.000    | 1.980 (1.350–2.903)  | 0.000   |
| Cytogenetic risks               | 1.723 (1.386–2.143)  | 0.000    | 1.427 (1.112–1.831)  | 0.005   |
| NPM1 mutations                  | 0.769 (0.371–1.594)  | 0.479    |                      |         |
| FLT3-ITD mutations              | 0.858 (0.396–1.860)  | 0.699    |                      |         |
| C-KIT mutations                 | 0.870 (0.319–2.375)  | 0.785    |                      |         |
| N/K-RAS mutations               | 1.097 (0.549–2.192)  | 0.793    |                      |         |
| DNMT3A mutations                | 1.615 (0.745–3.500)  | 0.225    |                      |         |
| U2AF1 mutations                 | 2.482 (0.771–7.995)  | 0.128    |                      |         |
| IDH1/2 mutations                | 0.844 (0.265–2.684)  | 0.774    |                      |         |
| SRSF2 mutations                 | 2.113 (0.767–5.820)  | 0.148    |                      |         |
| SETBP1 mutations                | 0.657 (0.091–4.729)  | 0.677    |                      |         |

Variables including LEP methylation (hypermethylation vs. non-hypermethylation), age (≤ 60 vs. > 60 years), WBC (≥ 30 × 10^9 vs. < 30 × 10^9 /L), and gene mutations (mutant vs. wild-type). Multivariate analysis includes variables with P < 0.100 in univariate analysis.
Table 5  Cox regression analyses of variables for overall survival in non-M3 AML patients

| Variables                  | Univariate analysis | Multivariate analysis |
|----------------------------|---------------------|-----------------------|
|                            | Hazard ratio (95% CI) | P value | Hazard ratio (95% CI) | P value |
| LEP methylation            | 1.496 (0.979–2.287)  | 0.063    | 1.584 (1.018–2.464)   | 0.041   |
| Age                        | 2.015 (1.362–2.981)  | 0.000    | 1.802 (1.203–2.700)   | 0.004   |
| WBC                        | 1.933 (1.302–2.870)  | 0.001    | 1.796 (1.209–2.668)   | 0.004   |
| Cytogenetic risks          | 1.525 (1.173–1.982)  | 0.002    | 1.388 (1.050–1.834)   | 0.021   |
| NPM1 mutations             | 0.646 (0.310–1.346)  | 0.243    |                      |        |
| FLT3-ITD mutations         | 0.905 (0.416–1.967)  | 0.801    |                      |        |
| C-KIT mutations            | 0.757 (0.239–2.402)  | 0.636    |                      |        |
| N/K-RAS mutations          | 0.944 (0.470–1.895)  | 0.871    |                      |        |
| DNMT3A mutations           | 1.420 (0.653–3.088)  | 0.377    |                      |        |
| U2AF1 mutations            | 2.293 (0.709–7.413)  | 0.166    |                      |        |
| IDH1/2 mutations           | 0.722 (0.226–2.309)  | 0.583    |                      |        |
| SRSF2 mutations            | 1.892 (0.685–5.222)  | 0.218    |                      |        |
| SETBP1 mutations           | 0.576 (0.080–4.152)  | 0.584    |                      |        |

Variables including LEP methylation (hypermethylation vs. non-hypermethylation), age (≤ 60 vs. > 60 years), WBC (≥ 30 × 10⁹ vs. < 30 × 10⁹/L), and gene mutations (mutant vs. wild-type). Multivariate analysis includes variables with P < 0.100 in univariate analysis.

Fig. 4 MicroRNA signatures correlated with LEP in AML. a Expression heatmap of differentially expressed microRNAs between lower- and higher-expressed LEP in AML patients among TCGA datasets (FDR < 0.05, P < 0.05 and |log2 FC| > 1). b Venn results of microRNAs which could target LEP predicted by TargetScan (http://www.targetscan.org/vert_72/), miRDB (http://mirdb.org/miRDB/), and miRWalk (http://mirwalk.umm.uni-heidelberg.de/). c Venn results of microRNAs shared in the negatively correlated microRNAs in a and the bioinformatics prediction in b.
that leptin presented an oncogenic role in AML biology by affecting cell proliferation and angiogenesis [29–31]. In clinics, although no significant difference of serum leptin concentrations were found between de novo AML patients and controls in two previous reports [32, 33], two independent investigations by Aref et al. and Bruserud et al. showed that serum leptin levels in AML patients were significantly lower than controls and had negative correlation with percentage of BM blasts and white blood cells [34, 35]. In our study, we detected LEP mRNA level in BMMNCs but not in serum of AML patients, and were found to be significantly decreased. The decreased expression of LEP may be caused by LEP promoter methylation in AML cells. In accordance with the previous study, we also observed LEP hypermethylation was associated with higher percentage of BM blasts and lower platelets. These results suggested DNA methylation-mediated leptin inactivation was a frequent event in AML cells. The reduction of autocrine of leptin in leukemia cells may negatively feedback regulates the increase of paracrine of leptin from adipose tissues into cancer microenvironment to promote leukemogenesis. Accordingly, further functional studies in vivo and in vitro are required to confirm our hypothesis.

Besides the DNA methylation, miRNAs expression was also identified to be associated with LEP expression in AML. In this study, we identified that LEP expression may be also regulated by miR-517a/b expression. Although few investigations revealed the miR-517a/b expression pattern in AML, a number of studies have reported the oncogenic role of miR-517a/b in diverse human solid tumors [36–38]. These results suggested that multiple factors were involved in regulating LEP expression in AML biology. Obviously, additional studies are required to confirm the direct links of LEP with miR-517a/b by luciferase assay, and the direct role of miR-517a/b in AML needs further functional studies.

Conclusion
Our findings indicated that the obesity-related gene LEP methylation is associated with LEP inactivation, and acts as an independent prognostic predictor in AML.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13148-021-01013-9.

Additional file 1. Primers used for MethylTarget sequencing, qPCR and qMSP.

Additional file 2. The impact of obesity-related genes expression on leukemia-free survival among AML patients from TCGA databases.

Additional file 3. Different expressed microRNAs between lower and higher LEP expression groups.

Additional file 4. Venn results of microRNAs targeting LEP.

Abbreviations
AML: Acute myeloid leukemia; ELN: European LeukemiaNet; BMI: Body mass index; TCGA: The Cancer Genome Atlas; qMSP: Real-time quantitative methylation-specific PCR; WHO: World Health Organization; BM: Bone marrow; BMMNCs: BM mononuclear cells; qPCR: Real-time quantitative PCR; CR: Complete remission; LFS: Leukemia-free survival; OS: Overall survival; CN-AML: Cytogenetically normal AML; FAB: French-American-British.

Acknowledgements
None.

Authors’ contributions
JZ and JQ conceived and designed the experiments, TZ and YG performed the experiments, JZ and ZX analyzed the data; WZ collected the clinical data; JQ, JZ, JL, JM, XW, ZD and WZ offer the technical and funding support, JZ wrote the paper. All authors read and approved the final manuscript.

Funding
The work was supported by National Natural Science Foundation of China (81900166, 81900163, 81970118, 81970156), Medical Innovation Team of Jiangsu Province (CXTDB2017002), Natural Science Foundation of Jiangsu Province for Youths (BK20180280), Zhenjiang Clinical Research Center of Hematology (SS2018009), Social Development Foundation of Zhenjiang (SH2017040, SH2018044, SH2019065, SH2019067, SH2020055), Scientific Research Project of The Fifth 169 Project of Zhenjiang (21), Youth Medical Talents Project of “Ke Jiao Qiang Wei” Project of Jiangsu Province (QNRZC2016450), Medical Field of Zhenjiang “Jin Shan Ying Cai” Project, Scientific Research Foundation of Affiliated People’s Hospital of Jiangsu University for Ph.D. (KFB202002).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate
The present study approved by the Ethics Committee and Institutional Review Board of the Affiliated People’s Hospital of Jiangsu University.

Consent for publication
Written informed consents were obtained from all enrolled individuals prior to their participation.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Department of Hematology, Affiliated People’s Hospital of Jiangsu University, 8 Dianli Rd., Zhenjiang 212002, People’s Republic of China. 2 Zhenjiang Clinical Research Center of Hematology, Zhenjiang, Jiangsu, People’s Republic of China. 3 The Key Lab of Precision Diagnosis and Treatment in Hematologic Malignancies of Zhenjiang City, Zhenjiang, Jiangsu, People’s Republic of China. 4 Laboratory Center, Affiliated People’s Hospital of Jiangsu University, 8 Dianli Rd., Zhenjiang 212002, People’s Republic of China.

Received: 28 March 2020 Accepted: 13 January 2021
Published online: 23 January 2021

References
1. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136–52.
2. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
7. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of US adults. N Engl J Med. 2003;348(17):1625–38.

8. Larsson SC, Wolk A. Overweight and obesity and incidence of leukemia: a meta-analysis. Int J Cancer. 2008;122(6):1418–21.

9. Orgeil, Genkniker JM, Aggarwal BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Gabriel S, Getz G, Sougnez C, Zou L, Leiserson MD, Vandin F, Wu HT, Wood CG, Wu X. Prognostic significance of promoter CpG island methylation of obesity-related genes in patients with nonmetastatic renal cell carcinoma. Cancer. 2017;123(18):3617–27.

10. Tavtigian SV, Puig GR, and Sangerian. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, and Cancer Genome Atlas. The cBio cancer genomics portal: an open platform for exploring cancer genomics data. Cancer Discov. 2012;2(5):401–4.

11. Arber DA, Orazi A, Massion PP, Holdener M, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 world health organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.

12. Zhou JD, Wang YX, Zhang TJ, Li X, Gu Y, Zhang W, Ma JC, Lin J, Qian J, and Zhou JD. Identification and validation of prognosis-related DLX5 methylation as an epigenetic driver in myeloid neoplasms. Clin Transl Med. 2020;10(2):e29.

Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.