IncRNA MVIH correlates with disease features, predicts treatment response and survival in pediatric acute myeloid leukemia

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Abstract

Objective: Long non-coding RNA microvascular invasion in hepatocellular carcinoma (Inc-MVIH) is correlated with unfavorable prognosis in several malignancies, while limittedly studied in pediatric acute myeloid leukemia (AML). This study aimed to investigate the correlation of Inc-MVIH with disease features, response to induction therapy, and survival in pediatric AML patients.

Methods: A total of 129 de novo pediatric AML patients who were retrospectively analyzed and 60 children with non-malignant hematological diseases who underwent bone marrow examination were reviewed as controls. Bone marrow mononuclear cells (BMMCs) were isolated from all participants to detect Inc-MVIH expression by reverse transcription-quantitative polymerase chain reaction. The complete remission status after 1 course of induction therapy, event-free survival, and overall survival of pediatric AML patients were recorded.

Results: Lnc-MVIH was upregulated in pediatric AML patients compared with controls (p < 0.001). In pediatric AML patients, Inc-MVIH was correlated with increased bone marrow blasts, less inv(16) or t(16;16) abnormality, and higher Chinese Medical Association (CMA) risk stratification (all p < 0.05), whereas its correlation with National Comprehensive Cancer Network (NCCN) risk stratification was not statistically significant (p = 0.098). As for prognosis, Inc-MVIH high expression patients presented with lower complete response rate to 1 course of induction therapy (61.5% vs. 79.7%, p = 0.024), shorter event-free survival (median 12.0 months vs. 22.0 months, p = 0.006), and overall survival (median 28.0 months vs. 42.0 months, p = 0.043) compared with Inc-MVIH low expression patients.

Conclusion: Lnc-MVIH correlates with poor treatment response and unfavorable survival in pediatric AML.

KEYWORDS
complete remission, Inc-MVIH, pediatric AML, risk stratification, survival
1 | INTRODUCTION

Pediatric acute myeloid leukemia (AML) is a hematological malignancy presenting as increased proliferation and attenuated differentiation of the hematopoietic progenitor cells. As the second most common childhood leukemia (around 25%), pediatric AML reaches a first peak incidence at 2 years old and the second peak at 16 years old. With the progression in risk stratification, anti-leukemic drugs, stem cell transplantation techniques, and supportive care over the past decades, the prognosis of pediatric AML has been largely improved with the survival rates reaching over 70%. However, the treatments containing intensive chemotherapy are also at high rates of both acute and chronic toxicity; moreover, around 50% of pediatric AML patients may experience relapse with poor response to treatment. Therefore, exploration of novel treatment targets based on the understanding of molecular pathologies is necessary to further improve the treatment outcome of pediatric AML.

As reported from leukemic studies, long non-coding RNA (lncRNA) involves in the pathogenesis of AML post-transcriptionally; for instance, the well-known lnc-HOTAIR is upregulated in AML and predicts poor prognosis. IncRNA microvascular invasion in hepatocellular carcinoma (lnc-MVIH) has been investigated in several solid tumors. For instance, Inc-MVIH is upregulated and correlates with unfavorable tumor features and poor prognosis in hepatocellular carcinoma, breast cancer, and non-small-cell lung cancer. However, Inc-MVIH is still novel to hematological malignancies, with only two relative studies in adult AML, which disclose that inhibiting Inc-MVIH suppresses AML progression, and Inc-MVIH predicts poor prognosis. For pediatric AML, neither the molecular mechanism nor the clinical implication of Inc-MVIH has been validated. In this study, we detected Inc-MVIH level in pediatric AML patients and investigated the correlation of Inc-MVIH with disease features, response to induction therapy, and survival in pediatric AML patients.

2 | MATERIALS AND METHODS

2.1 | Study population

This study retrospectively analyzed 129 de novo pediatric AML patients admitted to our hospitals from January 2016 to December 2019. The eligible patients were as follows: (1) diagnosed as pediatric AML according to the diagnosis criteria of Chinese Medical Association (CMA) based on clinical symptoms, hemogram, and bone marrow (BM) morphology; (2) age ≤16 years; (3) had complete records of clinical characteristics; (4) had clinical response records after 1 course of the standard induction therapy regimen; and (5) had frozen storage of BM specimen before initiation of treatment. The following patients were excluded: (1) M3 in French-American-Britain (FAB) classification; and (2) had history of other malignancies (eg, malignant hematological diseases or solid tumors). Besides, 60 children with non-malignant hematological diseases (eg, idiopathic thrombocytopenic purpura or megaloblastic anemia) who underwent BM examination in our hospitals at the corresponding period were reviewed as controls. All controls’ age and gender matched with pediatric AML patients and had available frozen storage of BM specimens.

The ethics committee approved this study, and written informed consent was provided by guardians of AML children and children with non-malignant hematological diseases.

2.2 | Risk stratification assessment

Basic characteristics, cytogenetics, and molecular genetics of pediatric AML patients were obtained from medical records. The risk stratification of pediatric AML patients was assessed using the National Comprehensive Cancer Network (NCCN) guidelines of AML and CMA guidelines of pediatric AML, respectively. According to NCCN guidelines of AML, risk stratification was classified as better risk, moderate risk, and poor risk based on cytogenetics and molecular genetics of pediatric AML patients. According to CMA guidelines of pediatric AML, the prognostic risk factors of pediatric AML patients included the following: (1) age ≤1 years at diagnosis; (2) WBC ≤100*10^9/L at diagnosis; (3) chromosome karyotype; (4) myelodysplastic syndromes (MDS)-AML; and (5) not acquired clinical remission after 1 course of standard induction therapy regimen. The risk stratification in CMA guidelines of pediatric AML was classified as follows: (1) low risk: M3, M2b, or M4 with eosinophilia patients or other FAB classification patients with inv(16); (2) moderate risk: other FAB classification patients apart from M3, M2b, or M4 with eosinophilia or patients without those prognostic risk factors described above; and (3) high risk: patients with any of those prognostic risk factors described above.

2.3 | BM specimen collection and detection

The frozen stored BM specimens of pediatric AML patients and controls (whose sample collected was done during BM examination) were acquired from pathology department. Then, the bone marrow mononuclear cells (BMMCs) were separated from BM specimens using density gradient centrifugation. The expression of Inc-MVIH in BMMCs was detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The following kits were used in RT-qPCR for total RNA extraction, reverse transcription to cDNA and qPCR, and amplification/detection, respectively: TRIzol™ Reagent (Thermo Fisher Scientific, Waltham, Massachusetts, USA), iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, California, USA), and QuantiNova SYBR Green PCR Kit (Qiagen, Dusseldorf, Nordrhein-Westfalen, German). The procedures of RT-qPCR and the design of primer in RT-qPCR were carried out according to a study published previously. GAPDH was used as the internal reference, and the result was calculated.
using $2^{-\Delta\Delta Ct}$ method. The primers were listed as follows: Inc-MVIH forward primer: 5′-AATTTTGACATCTGACACGAC-3′, reverse primer: 5′-TTCAAAATCCCACTAGCCCA-3′; GAPDH forward primer: 5′-TGACCACAGTCCATGCCATCAC-3′, reverse primer: 5′-TTCAAAATCCCACTACGCCCA-3′.

2.4 | Treatment and remission assessment

All pediatric AML patients received anyone of standard induction therapy regimens below: (1) DAE (daunorubicin, Ara-C, and etoposide): day 1–3, daunorubicin (DNR) 40 mg/(m$^2$/d), intravenous injection (iv) drip; day 1–7, cytosine arabinoside (Ara-C) 200 mg/(m$^2$/d), q12 h, subcutaneous injection; day 5–7, etoposide 100 mg/(m$^2$/d), iv drip; (2) HAD (homoharringtonine, Ara-C, daunorubicin): day 1–7, homoharringtonine (HRT) 3 mg/(m$^2$/d), iv drip; day 1–7, Ara-C 200 mg/(m$^2$/d), q12 h, subcutaneous injection; day 1–3, DNR 40 mg/(m$^2$/d), iv drip; and (3) IA (idarubicin, Ara-C): day 1–3, idarubicin (IDA) 10 mg/(m$^2$/d), iv drip; day 1–7, Ara-C 200 mg/(m$^2$/d), q12 h, subcutaneous injection. After 1 course of the standard induction therapy regimen, the patients’ clinical remission status was assessed.14 For all patients, after 1 course of the standard induction therapy regimen, the subsequent therapy was carried out referring to the guidelines of AML based on their clinical remission status.14 The disease status and survival status of patients were extracted from follow-up records. Patients were followed up from January 2016 to December 2019 (duration: 1–46 months). Event-free survival (EFS) was calculated from initiation of treatment to the date of induction therapy failure, or relapse from CR or death. Overall survival (OS) was calculated from initiation of treatment to the date of death. Lost follow-ups were analyzed according to the last follow-up results.

2.5 | Statistical analysis

SPSS 22.0 (IBM, Chicago, IL, USA) was used to perform statistical analysis. GraphPad Prism 7.00 (GraphPad Software, San Diego, California, USA) was used to plot figures. Data were used for distribution using the Shapiro-Wilk test. Normally distributed continuous variables were shown as mean with standard deviation (SD), and skewed distributed continuous variables were displayed as median with range or interquartile range (IQR). Categorical variables were summarized as frequency and percentage (No. [%]). Wilcoxon rank sum test was used to compare the difference in skewed distributed continuous variables or gradational categorical variables between two groups. Student’s t test was used to analyze the difference of normally distributed continuous variables between two groups. The chi-square test or Fisher’s exact test was used to determine the difference of non-gradational categorical variables between two groups. The Kaplan-Meier curve was used to display EFS and OS, and the difference in EFS and OS between two groups was determined by the log-rank test. $p$ value $< 0.05$ was considered significant.

### Table 1: Basic characteristics of pediatric AML patients

| Items                         | Pediatric AML patients (N = 129) |
|-------------------------------|----------------------------------|
| Age (years), mean ± SD        | 6.8 ± 2.9                        |
| Gender, No. (%)               |                                  |
| Female                        | 63 (48.8)                        |
| Male                          | 66 (51.2)                        |
| Height (cm), mean ± SD        | 118.7 ± 19.0                     |
| Weight (kg), mean ± SD        | 24.0 ± 9.0                       |
| FAB classification, No. (%)   |                                  |
| M1                            | 5 (3.9)                          |
| M2                            | 56 (43.4)                        |
| M4                            | 27 (20.9)                        |
| M5                            | 41 (31.8)                        |
| WBC (*10$^3$/L), median (range)| 30.4 (2.0–465.6)                 |
| BM blasts (%), mean ± SD      | 70.8 ± 16.8                      |
| NCCN risk stratification, No. (%)|                                 |
| Better                        | 35 (27.1)                        |
| Moderate                      | 44 (34.1)                        |
| Poor                          | 50 (38.8)                        |
| CMA risk stratification, No. (%)|                                 |
| Low                           | 24 (18.6)                        |
| Moderate                      | 56 (43.4)                        |
| High                          | 49 (38.0)                        |

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CMA, Chinese Medical Association; FAB, French-American-Britain; NCCN, National Comprehensive Cancer Network; SD, standard deviation; WBC, white blood cell.

3 | RESULTS

3.1 | Pediatric AML patients’ characteristics

The pediatric AML patients were in mean age of 6.8 ± 2.9 years, and female/male ratio was 63 (48.8%)/66 (51.2%). Number of patients at FAB classification M1, M2, M4, and M5 was 5 (3.9%), 56 (43.4%), 27 (20.9%), and 41 (31.8%), respectively. Besides, under NCCN risk stratification, 35 (27.1%), 44 (34.1%), and 50 (38.8%) patients were at better, moderate, and poor risk, respectively; while under CMA risk stratification, 24 (18.6%), 56 (43.4%), and 49 (38.0%) patients were at low, moderate, and high risk, respectively. Other characteristics including height, weight, WBC, and BM blasts level are listed in Table 1. Cytogenetic and molecular genetic information of patients are listed in Table 2.

3.2 | Correlation of Inc-MVIH with disease features of pediatric AML patients

The expression of Inc-MVIH was higher in pediatric AML patients compared with controls ($p < 0.001$) (Figure 1). In pediatric
AML patients, Inc-MVIH was correlated with higher BM blasts ($p = 0.017$), and less inv(16) or t(16;16) abnormality ($p = 0.014$) (Table 3). Further analysis indicated that Inc-MVIH high expression was correlated with higher CMA risk stratification ($p = 0.024$) (Figure 2B) but not correlated with NCCN risk stratification ($p = 0.098$) (Figure 2A).

### 3.3 | Correlation of Inc-MVIH with treatment response to induction therapy in pediatric AML patients

The CR rate after 1 course of standard induction therapy in total pediatric AML patients was 70.5%. Further comparison revealed that CR rate was lower in Inc-MVIH high (CR: 61.5%) expression patients compared with Inc-MVIH low (CR: 79.7%) expression patients ($p = 0.024$) (Figure 3), indicating that Inc-MVIH was...
3.4 | Correlation of Inc-MVIH with survival in pediatric AML patients

The EFS in Inc-MVIH high expression patients (median EFS: 12.0 months, 95% CI: 8.9–15.1 months) was shorter compared with that in Inc-MVIH low expression patients (median EFS: 22.0 months, 95% CI: 7.1–36.9 months) \( (p = 0.006) \) (Figure 4A). Besides, the OS in Inc-MVIH high expression patients (median OS: 28.0 months, 95% CI: 23.6–32.4 months) was also reduced compared with that in Inc-MVIH low expression patients (median OS: 42.0 months, 95% CI: 26.0–58.0 months) \( (p = 0.043) \) (Figure 4B). These indicated that Inc-MVIH was correlated with shorter EFS and OS in pediatric AML patients.

4 | DISCUSSION

By analyzing Inc-MVIH in pediatric AML, we observed that: (a) Inc-MVIH was upregulated in pediatric AML patients compared with controls; (b) in pediatric AML patients, Inc-MVIH expression was correlated with increased BM blasts, less inv(16) or t(16;16) abnormality, and higher CMA risk stratification; and (c) Inc-MVIH was correlated with unfavorable treatment response to induction therapy and survival.

LncRNAs underlies the fundamental biological processes, and their dysregulations are found in tumorigenesis of a variety of malignancies. \(^8\) Inc-MVIH, as previously reported, is a cancer-related lncRNA that is initially found to be upregulated in HCC. \(^7\) It promotes tumor growth and predicts poor recurrence-free survival in HCC patients. \(^7\) In NSCLC, Inc-MVIH is correlated with larger tumor size, lymph node metastasis, and unfavorable survival. \(^8\) Relative studies of Inc-MVIH also report its tumor-promoting role or predictive potential for poor prognosis in other solid tumors including breast cancer, pancreatic ductal adenocarcinoma, gastric cancer, and glioma. \(^10\)–\(^12\) While for hematological malignancies, only limited evidence about Inc-MVIH function is available. A mechanical exploration reveals from cellular level that Inc-MVIH is overexpressed in adult AML cell lines, and inhibition of Inc-MVIH suppresses cell proliferation, and promotes cell apoptosis via microRNA-505–mediated high-mobility group box 1 (HMGB1) and cyclin E2 (CCNE2). \(^6\) As for the clinical findings, only one study exhibits that Inc-MVIH level is increased in adult AML patients compared with controls and correlates with worse risk stratification. \(^13\) Although Inc-MVIH has been studied in adult AML, evidence about its oncogenic role in pediatric AML is still lacking. Therefore, we retrospectively analyzed Inc-MVIH in pediatric AML and observed that Inc-MVIH was upregulated with pediatric AML patients compared with controls, which was in accordance with the findings in adult AML. In addition, Inc-MVIH high expression was correlated with increased BM blasts, less inv(16) or t(16;16) abnormality, and higher CMA risk stratification in pediatric AML patients. This could be explained by evidence from the previous study that (1) Inc-MVIH promoted cell proliferation via regulating microRNA-505 and the subsequent oncogenes (HMGB1 and CCNE2) in leukemic cells. \(^6\) Therefore, Inc-MVIH was correlated with increased BM blasts, which was indicative for more advanced disease severity. (2) A previous mRNA sequencing showed that differentially expressed genes by Inc-MVIH up-/down-regulation is involved in cell cycle arrest, and Inc-MVIH was correlated with chromatin remodeling as well as inactivation. \(^9\) Therefore, Inc-MVIH might interfere with clonal composition of inv(16) or t(16;16) abnormality in pediatric
The clinicopathological observations of lnc-MVIH was similar to a factor predicting poor prognosis; thus, lnc-MVIH was correlated with NCCN risk stratification. However, further investigation was needed to verify this.

The prognostic value of lnc-MVIH has been confirmed in solid tumors, and the previous study in adult AML has also disclosed that it is negatively correlated with CR rate, poor EFS, and OS. In our study, we further analyzed the correlation of lnc-MVIH with prognosis of pediatric AML patients and disclosed that lnc-MVIH was indicative for poor CR rate to standard induction therapy and survival. Our findings were consistent with the previous evidence in adult AML, which could be due to that: (1) the pathogenesis of pediatric and adult AML that lnc-MVIH involved in shared similarities, thus, the clinicopathological observations of lnc-MVIH was similar; (2) lnc-MVIH increased cell proliferation and reduced apoptosis in adult AML, and it was correlated with higher BM blasts, which suggested that lnc-MVIH accelerated disease progression and increased disease severity, thereby shortening survival of pediatric AML patients; and (3) lnc-MVIH was correlated with less inv(16) or t(16;16) abnormality, which was indicative for unfavorable prognosis; in addition, it was correlated with higher CMA risk stratification, which was also a factor predicting poor prognosis; thus, lnc-MVIH was correlated with worse survival in pediatric AML patients.

Our study initially revealed the role of lnc-MVIH in pediatric AML, whereas limitations still existed. (1) Although the molecular mechanism of lnc-MVIH in adult AML was previously revealed, validation was still lacking in pediatric AML. (2) Considering the heterogeneity of pediatric AML, the results of this study should be further assessed in a larger and more diverse population. In addition, the number of controls should be expanded to match the patients’ number. (3) As an observational study, there might be bias; for instance, the interventions might influence the prognosis of patients; thus, prospective investigation with larger sample size was needed to validate our findings.

In summary, lnc-MVIH correlates with increased BM blasts, less inv(16) or t(16;16) abnormality, higher CMA risk stratification, and poor treatment response/survival profiles in pediatric AML patients.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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