EBioMedicine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

The Long Noncoding RNA MEG3 and its Target miR-147 Regulate JAK/STAT Pathway in Advanced Chronic Myeloid Leukemia

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ARTICLE INFO

Article history:
Received 17 April 2018
Received in revised form 12 July 2018
Accepted 12 July 2018
Available online xxxx

Keywords:
lncRNA MEG3
Chronic myeloid leukemia blast crisis
Chidamide
miR147 and epigenetic

ABSTRACT

Background: Long non-coding (lnc) RNAs plays an important role in chronic myeloid leukemia (CML). In this study, we aimed to uncover the mechanism of the lncRNA maternally expressed 3 (MEG3) and its target microRNA-147 (miR-147) in CML.

Methods: Sixty CML patients and 10 healthy donors were included in the study. The methylation of MEG3 and miR-147 promoter was determined by methylation-specific PCR. The relationship of MEG3 and miR-147 was explored by luciferase assay. The interactions of proteins were studied by RNA pull-down assay, RNA immunoprecipitation and co-immunoprecipitation.

Findings: Patients in accelerated phase CML (CML-AP) and blast phase CML (CML-BP) showed lower expressions of MEG3 and miR-147 and higher expressions of DNMT1, DNMT3B, MBD2, MECP2 and HDAC1 compared to the controls. These patients also showed a higher degree of methylation of MEG3 and miR-147 while there was a reduction after chidamide treatment. Furthermore, the overexpression of MEG3 and miR-147 inhibited cell proliferation both *in vivo* and *in vitro*, promoted apoptosis and decreased the expressions of DNMT1, DNMT3A, DNMT3B, MBD2, HDAC1 and MECP2. We also found MEG3 interacted with DNMT1, JAK2, STAT3, HDAC1, and TYK2, and JAK2 was bound to STAT3, STAT5 and MYC. More interestingly, JAK2 was bound to TYK2 by the bridge of MEG3.

Interpretation: LncRNA MEG3 and its target miR-147 may serve as a novel therapeutic target for CML blast crisis, and chidamide might have a potential clinical application in treating CML blast crisis.

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https://doi.org/10.1016/j.ebiom.2018.07.013

Research in Context

Evidence before this Study

Chronic myeloid leukemia (CML) is a bone marrow clone malignancy. In clinic, tyrosine kinase inhibitors (TKIs) were primarily chosen for CML treatment, while some patients showed resistance to or cannot tolerate TKI administration. Therefore, it is urgent to explore novel therapeutic targets or potential medication. Development of CML is a complex process, many molecules play important roles, including long non-coding RNA (lncRNA) and microRNA (miRNA). It has been shown that lncRNA maternally expressed 3 (MEG3) can inhibit cancer cell proliferation and is associated with poor prognosis of cancer patients. MEG3 can inhibit cell proliferation in CML. MEG3 may be an important molecule in the progression of diseases including CML, while the regulation mechanism was not clear. MiR147, which is downregulated in colon cancer, suggesting that miR147 can act as tumor suppressor. The role of miR-147 in CML was unknown. In the study, we aimed to explore the role and regulation mechanism of MEG3 and miR1-47 in CML development to explore the novel therapeutic targets.Added Value of this Study

MEG3 may be an important molecule in the progression of CML. In the study, we explored the regulation mechanism of MEG3 on CML progression. Chidamide is a novel histone deacetylase inhibitor to treat cutaneous T-cell lymphoma. In this study, we detected the epigenetic regulation of MEG3 and regulation mechanism to further uncover the pathology of CML blast crisis, and the potential treatment effect of chidamide on CML blast crisis.Implications of all the Available Evidence

We found in our study that lncRNA MEG3 and its target miR-147 may serve as a novel therapeutic target for CML blast crisis, and chidamide might also have a potential clinical application in treating CML blast crisis.

1. Introduction

Chronic myeloid leukemia (CML) is a bone marrow clone malignancy characterized by the formation of the breakpoint cluster region (BCR) and Abelson murine leukemia (ABL) fusion gene that encodes BCR-ABLp210 [1, 2]. The pathology of CML comprises three phases, namely chronic phase (CML-CP), accelerated phase (CML-AP), and blast phase (CML-BP). The CML-AP and CML-BP are the hallmarks of advanced CML [3]. In clinic, tyrosine kinase inhibitors (TKIs) were primarily chosen for CML treatment, which improves the overall survival and the five-year progression-free survival of CML patients [4]. However, some patients showed resistance to or cannot tolerate TKI administration [5]. Therefore, it is urgent to explore novel therapeutic targets or potential medication.

Development of CML is a complex process, many molecules play important roles, including long non-coding RNA (lncRNA) [6]. For example, a study showed that lncRNA maternally expressed 3 (MEG3) can inhibit cell proliferation of CML [7]. Specifically, MEG3 can inhibit cancer cell proliferation by binding to the PRC2 complex, and low levels of MEG3 were associated with a poor prognosis of cancer patients [8]. Moreover, the function of MEG3 was also reported in many cancer cells lines [9, 10]. Therefore, MEG3 may be an important molecule in the progression of diseases including CML. Therefore, in the study, we explored the regulation mechanism of MEG3 on CML progression. Besides lncRNAs, microRNAs (miRNAs) can also regulate the progression of diseases [11–14].

In addition to molecules, many genetic and molecular biological events were involved in the development of diseases including epigenetic regulation [15]. Epigenetics are the study of heritable changes in gene expression, without any alteration of the DNA sequence [16]. The abnormal methylation of promoter regions plays an important role in tumorigenesis, including human choriocarcinoma and squamous cell lung cancer [17, 18]. Furthermore, the study of lncRNA-associated methylation has gained increased attention [19, 20].

In the cells where methylation regulation occurs, the expressions of methylation related genes were significantly changed, such as DNMT1, DNMT3A, DNMT3B, MBD2, HDAC1, and MECP2. Histone deacetylases (HDACs) also involved epigenetic regulation. HDAC expression was increased in the majority of tumor cells and induces tumorigenesis. HDAC inhibitors (HDACis) are a novel family of drugs that achieved positive results in the treatment of hematological malignancies [21–23] and certain solid tumors [24]. HDACis interfere with the function of HDACs and reverse the effect of their overexpression [25, 26]. Chidamide is a novel HDACi that is approved to treat cutaneous T-cell lymphoma [26]. However, whether chidamide influences the epigenetic regulation of MEG3 and miR-147 in KCL22 and K562 cells remains largely unknown.

Therefore, we aimed to detect the epigenetic regulation of MEG3 and regulation mechanism to further uncover the pathology of CML blast crisis, and the potential treatment effect of chidamide on CML blast crisis in the study.

2. Materials and Methods

2.1. Collection of Bone Marrow Samples

The bone marrow samples of 60 CML patients and 10 healthy donors were collected at Department of Hematology of the Second Hospital of Hebei Medical University between May 2016 and June 2017 (Table 1). The bone marrow samples from 10 healthy donors were used as controls. In addition, we collected 3 CML-AP patients and 3 CML-BP patients to study the role of chidamide for treatment of CML. Peripheral blood mononuclear cells were isolated by lymphocyte separation. The inclusion criteria of patients were as follows: (i) diagnosis of CML via bone marrow morphology, immunology, molecular biology, and cytogenetic analyses; (ii) clear pathological staging; and (iii) availability of intact clinical data. The exclusion criteria were as follows: (i) significant organ dysfunction; (ii) pregnancy; and (iii) failure to provide informed consent. No chemotherapy was administered before the collection of the specimens. The study was approved by the Ethics Committee of the Department of Hematology of the Second Hospital of Hebei Medical University, and each patient signed an informed consent.

Table 1
Characteristics of the patients included in the study.

Item	CML-CP (n = 30)	CML-AP (n = 15)	CML-BP (n = 15)
Age (years), median (range)	41.4(9–65)	49.1(13–69)	51.9(20–69)
Male/female, (n/n)	20/10	9/6	10/5
WBCs × 10^9/median (range)	221.4 (30.2–517)	263.5 (47.4–396)	69.5 (27.4–224)
Hemoglobin level (g/l)	94(76–120)	75(61–105)	62.4(52–79)
Platelet count,10^9/median (range)	518(99–809)	305(52–725)	35.5(19–71)

AP, accelerated phase; BP, blast phase; CML, chronic myeloid leukemia; CP, chronic phase; WBC, white blood cells.

Please cite this article as: Li, Z., et al., The Long Noncoding RNA MEG3 and its Target miR-147 Regulate JAK/STAT Pathway in Advanced Chronic Myeloid Leukemia, EBioMedicine (2018), https://doi.org/10.1016/j.ebiom.2018.07.013