Biomedicine & Pharmacotherapy 100 (2018) 509–520

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Low expression of GFI-1 Gene is associated with Panobinostat-resistance in acute myeloid leukemia through influencing the level of HO-1

Bingqing Cheng[a,b], Sishi Tang[a,c], Nana Zhe[a,d], Dan Ma[a], Kunlin Yu[a,b], Danna Wei[a], Zheng Zhou[a,e], Tingting Lu[a], Jishi Wang[a,**], Qin Fang[c,e,*]

[a] Department of Hematology, Affiliated Hospital of Guizhou Medical University, Laboratory of Hematopoietic Stem Cell Transplantation Centre of Guizhou Province, Guiyang, Guizhou, China
[b] College of Pharmacy, Guizhou Medical University, Guiyang, Guizhou, China
[c] Department of Pharmacy, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China
[d] Department of Hematology, The First People's Hospital of Zunyi, Zunyi, Guizhou, China
[e] Department of Pharmacy, Affiliated Baiyun Hospital of Guizhou Medical University, Guiyang, Guizhou, China

ARTICLE INFO

Keywords:
GFI-1
HO-1
AML
Panobinostat
Chemotherapy-resistant
Akt

ABSTRACT

To improve the treatment outcomes of acute myeloid leukemia (AML), epigenetic modification has been widely tested and used in recent years. However, drug-resistance is still a choke point to cure the malignancy. The growth factor independent 1 transcriptional repressor (GFI-1), as a zinc-finger transcriptional repressor, can bind histone deacetylases to allow the transcriptional repression. According to the finding of our study, AML patients with low level of GFI-1 not only implicated poor prognosis but also caused Panobinostat-resistance. In our prevent study revealed that heme oxygenase-1(HO-1) was one of the main factors leading to chemotherapy sensitivity to AML. Thus, this study tried to test the correlation between GFI-1 and HO-1. Our study discovered that AML patients with lower expression of GFI-1 had higher level of HO-1, HDAC1, HDAC2 and HDAC3, which resulted in poor prognosis in AML. The results of the in vitro study were the same. Panobinostat is a promising new class of anti-cancer drugs in AML. However, knocking down GFI-1 by siRNA could eliminate the Panobinostat-induced cell apoptosis. Subsequently, we utilized ZnPP to down regulate the level of HO-1, finding that the Panobinostat-resistance between the low level of GFI-1 and empty vector had eased. After further exploring the mechanism, it could be found that with knock down GFI-1, the phosphorylation of Akt and PI3K could be activated. Subsequently, Akt pathway and HO-1 inhibitor were utilized respectively and the resistance was reversed. It suggested that the resistance of Panobinostat to AML cells at low level of GFI-1 was mainly due to up-regulated level of HO-1 through the PI3K-Akt pathway.

1. Introduction

The differentiation of hematopoietic stem cells into mature blood cells is a highly ordered process which, when disordered, causes leukemogenesis [1]. AML is a clonal malignant proliferative disease of the myeloid lineage of the hematopoietic system. It is well-known for refractory nature and poor prognosis [2,3]. In AML, leukemia stem cells (LSCs) play important roles in leukemia initiation and progression, also contributing to chemotherapeutic drug resistance and disease relapse [4]. Therefore, it is feasible to treat AML by controlling the differentiation of LSCs. Since acute promyelocytic leukemia was successfully treated by all-trans retinoic acid in 1987 [5], researchers have endeavored to induce the differentiation of leukemia cells by developing new drugs such as retinoic acid and vitamin D derivatives, Cytarabine, and histone deacetylase inhibitors (HDACis) [6–11]. However, these chemotherapeutic drugs often suffer from resistance or intolerance. Thus, finding alterative targets and additional therapeutic strategies for AML is of great significance.

In leukemia, monocyte differentiation is accompanied by classic transcription factor expression. Transcription factor-driven non-neoplastic mononuclear cell differentiation is a transcription-inducing factor for leukemia [6,12]. Growth factor independent 1 transcriptional repressor(GFI-1) is located on P-arm of chromosome 1 as a transcriptional repressor and plays an important role in the proliferation,

* Corresponding author at: Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, 550004, China.
** Corresponding author at: Guizhou Province Laboratory of Hematological Disease Diagnostic & Treat Centre, Department of Hematology, Affiliated Hospital of Guizhou Medical University, Guizhou Province Hematopoietic Stem Cell Transplantation Centre, Guiyang, Guizhou, 550004, China.
E-mail addresses: wangjishi9646@163.com (J. Wang), fq_fangqin@sina.com (Q. Fang).

https://doi.org/10.1016/j.biopha.2018.02.039
Received 27 October 2017; Received in revised form 11 February 2018; Accepted 13 February 2018

apoptosis and differentiation of hematopoietic stem cells in AML [13–18]. Decrease in GFI-1 expression suggests an obstacle to hematopoietic quiescence in leukemic bone marrow [19]. Epigenetic studies have shown that GFI-1 bound HDACs to allow transcriptional repression. GFI-1 has been associated with HDAC1, HDAC2 and HDAC3 [20–22]. The gene encodes a nuclear zinc finger protein that functions as a transcriptional repressor. This protein participates in diverse developmental contexts, including hematopoiesis and oncogenesis. It functions as part of a complex along with other cofactors to control histone modifications that lead to silencing of the target gene promoters, but the exact mechanism remains elusive [18,23]. Besides, GFI-1 is a novel index of poor prognosis in MDS and AML [24,25]. Low expression of GFI-1 may result in resistance to HDACis, probably because decreased GFI-1 enhances the acetylation of target gene, leading to active expressions of genes promoting the progression of myeloid malignancies. However, the underlying mechanism is still unclear [24].

HDACis have recently been regarded as a novel therapeutic modality for relieving tumor resistance [26,27]. HDAC enzymes are grouped into Class I, Class II, Class III and Class IV [28]. Panobinostat is a representative Class I HDACi [29,30]. We found that low expression of GFI-1 also caused AML cells to be insensitive to Panobinostat.

As a key protein upon oxidative stress response, HO-1 is mainly responsible for promoting cell protection, resisting apoptosis and alleviating inflammation [31,32]. Overexpression of HO-1 in various tumor tissues leads to tumor proliferation, apoptosis and angiogenesis, release of tumor-associated inflammatory cytokines, and drug resistance of solid tumors. Drug resistance and poor prognosis have been closely related. We hypothesized that GFI-1 affected the resistance to differentiation drugs, because differentiation is accompanied by the expressions of classic transcription factors. We have previously related high HO-1 expression with the drug resistance and proliferation of AML [31,33,34]. Nevertheless, the correlation between GFI-1 and HO-1 has not been reported hitherto.

In this study, we focused on the effect of low GFI-1 expression on the adverse prognosis of AML. We further explored whether GFI-1 was correlated with HO-1 upon chemotherapeutic response and the underlying mechanism.

2. Materials and methods

2.1. Reagents and antibodies

Panobinostat was obtained from Selleck Chemicals (Houston, Texas, USA). LY294002 was purchased from Beyotime Biotechnology (Shanghai, China). Antibodies against HO-1, β-actin, PI3K, PI3K-P85, Akt, P-Akt were purchased from Cell Signaling Technology (MA, USA). Antibodies against GFI-1, Bad, Bcl-2 and Bax were brought from Santa Cruz (Heidelberg, Germany).

2.2. Cell lines and cell culture

Human myeloid leukemia cell lines Kasumi-1 and HL-60 were obtained from Laboratory of Hematology, Affiliated Hospital of Guiyang Medical University. These cell lines were cultured at 37 °C in a 5% humidified atmosphere in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco BRL, MD, USA), penicillin (100 units/mL) and streptomycin (100 μg/mL).

2.3. Patient samples

Bone marrow samples were obtained from 64 newly diagnosed AML patients in Affiliated Hospital of Guiyang Medical University (Guiyang, Guizhou Province, China) between October 2015 and January 2018(Table 1). Patients with AML M3 were excluded. All samples contained ≥80% leukemia cells. Primary AML samples were isolated from marrow samples from consenting patients with AML. AML cells were isolated by Percoll density centrifugation. Unless otherwise noted, primary normal hematopoietic cells refer to normal mononuclear cells obtained from healthy consenting volunteers donating peripheral blood stem cells for allogeneic stem cell transplantation after G-CSF mobilization. Institutional Review Board approval was obtained from Affiliated Hospital of Guiyang Medical University (Guiyang, Guizhou Province, China). The study was performed in accordance with the modified Helsinki Declaration, and the protocol was approved by our ethical review board before study initiation. Informed consent was obtained from the patients and healthy volunteers.

2.4. siRNA transfection

siRNA against GFI-1 (si-GFI-1) was used to inhibit endogenous GFI-1 expression. Scrambled siRNA (si-NC) was used as a negative control. The siRNA sequences were designed and synthesized by Viewsolid Biotech Co., Ltd. (Beijing, China).

si-GFI-1: 5′-CCAGACUAUUCCCUCCGCCTTAACGGAGGGAAUAGUC UGGTT-3′

The final concentration of each siRNA was 0.13 pmol. Cells were electroporated using transfection buffer and Soly-fecter according to the manufacturer's instructions (Viewsolid Biotech Co., Ltd., Beijing, China). Forty-two hours after the initiation of transfection, the cells were harvested for quantitative real-time polymerase chain reaction (qRT-PCR) or panobinostat treatment. Western blot was performed after the transfected cells were cultured for 48 h.

2.5. In vitro proliferation assay

Cells were seeded at a density of 4000/well in 96-well plates. After overnight incubation, the cells were treated for 24, 48, and 72 h respectively with UA at different concentrations (0, 0.5, 1, 2, 4, and 8 μmol/l). The inhibitory effect of UA was determined using the cell counting kit-8 (CCK-8) assay (Beyotime Institute of Biotechnology, Haimen, China). Survival rate (SR) was determined using the following equation: SR (%) = (A Treatment /A Control) × 100%. The concentration that produced 50% cytotoxicity (IC50) was determined using GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA).

2.6. Apoptosis assay

Apoptosis was determined by annexin V-FITC and propidium iodide double staining according to the manufacturer's instructions (7sea biotech, Shanghai, China). Data were collected on a FACS Calibur flow cytometer (BD, San Jose, CA, USA). Results represent the mean of 3 independent experiments.

2.7. RNA isolation and qRT-PCR

Total RNAs from cell lines and primary mononuclear cell samples were extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. qRT-PCR was performed using an SYBR Green PCR Master Mix (TianGen Biotech, Beijing, China) and a PRISM 7500 real-time PCR detection system (ABI, USA). The levels of gene expression were analyzed relative to that of β-actin gene transcript.

The sequences for qRT-PCR were:

GFI-1-F 5′–GCAAGGCATTCAGCCAGAG-3′
GFI-1-R 5′–AAGGCAAAGGAGGAGCAA-3′
β-actin-F 5′-GAGACCTTCAACACCCCAGC-3′
β-actin-R 5′-ATGTCACGCACGATTTCCC-3′
HO-1-R 5′-ACCCATGACACCAAGGACCAGA-3′
HO-1-F 5′-GTGTAAGGACCCATCGGAGAAGC-3′
HDAC1-R 5′-CTTTGTGAGGGCGATAGA-3′
HDAC1-F 5′-CTACTACGACGGGGATGTT-3