Cancer Letters 405 (2017) 73–78

Contents lists available at ScienceDirect

Cancer Letters

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

Original Article

Efficacy of the CDK inhibitor dinaciclib *in vitro* and *in vivo* in T-cell acute lymphoblastic leukemia

Sausan A. Moharram [a, b], Kinjal Shah [a, b], Fatima Khanum [a, b], Alissa Marhäll [a, b], Mohiuddin Gazi [a, b], Julhash U. Kazi [a, b, *]

[a] Division of Translational Cancer Research, Department of Laboratory Medicine, Lund University, Lund, Sweden
[b] Lund Stem Cell Center, Department of Laboratory Medicine, Lund University, Lund, Sweden

ARTICLE INFO

Article history:
Received 20 June 2017
Received in revised form
17 July 2017
Accepted 19 July 2017

Keywords:
T-ALL
CDK inhibitor
Apoptosis
Cell cycle

ABSTRACT

T-cell acute lymphoblastic leukemia (T-ALL) is a heterogeneous disease of the blood affecting children, adolescents and adults. Although current treatment protocols for T-ALL have improved overall survival, a portion of T-ALL patients still experiences treatment failure. Thus, the development of novel therapies is needed. In this study, we used several patient-derived T-ALL cell lines to screen for an effective drug for T-ALL. Using a panel of 378 inhibitors against different kinases, we identified the CDK inhibitor dinaciclib as a potential drug for T-ALL. Dinaciclib treatment significantly reduced cell viability and completely blocked colony formation. Furthermore, cells treated with dinaciclib showed decreased expression of several pro-survival proteins including survivin, cyclin T1 and c-MYC. Dinaciclib treatment also increased accumulation of cells in G2/M phase and significantly induced apoptosis. Finally, dinaciclib extended survival of mice in a T-ALL cell xenograft model. Collectively, these data suggest that the CDK inhibitor dinaciclib is an active drug for T-ALL in the preclinical settings.

Introduction

Acute lymphoblastic leukemia (ALL) is a group of aggressive hematologic disorders affecting a wide range of the population including children, adolescents, and adults. Despite significant progress in the treatment of ALL, approximately 20% of pediatric and 50% of adult T-cell ALL (T-ALL) patients experience treatment failure [1]. After relapse, T-ALL patients receive intensive chemotherapy and stem cell transplantation. However, survival of this group of patients still remains poor. Therefore, the development of novel targeted therapies is likely to improve the survival. Genomic profiling and next generation sequencing have enhanced our understanding of the major genetic alterations in T-ALL [2]. The most common features include mutations in the transmembrane receptor NOTCH1 and the E3 ubiquitin ligase FBXW7. More than 50% of T-ALL patients harbor activating NOTCH1 mutations resulting in constitutive NOTCH signaling [3], and thus targeting NOTCH1, or its associated downstream pathway, is an attractive area of drug development. Mutations in FBXW7 are not as common as in NOTCH1 and are found in around 9–12% of patients with T-ALL [4,5].

Another class of dominant genetic aberrations includes the 9p21 deletion. This genetic alteration occurs in 40%–60% of T-ALL patients and affects the tumor suppressor genes CDKN2A and CDKN2B. CDKN2A and CDKN2B are important regulators of the cell cycle where the corresponding proteins act as inhibitors of cyclin-dependent kinases (CDKs) [6]. Therefore, loss of CDKN2A and CDKN2B genes accelerate the cell cycle, resulting in uncontrolled cell proliferation. Other genetic alterations include activating or inactivating mutations of several transcription factors, epigenetic factors, PTEN and several kinases [7]. Overexpression of the transcription factors TAL1 and TLX1 in combination with other genes accelerates T-ALL progression [8,9]. Loss of PTEN and activating mutations in kinases result in hyperactivation of several signaling cascades including the PI3K/AKT pathway, the RAS/MAPK pathway and the JAK/STAT pathway. Thus, inhibitors against specific signaling pathways can be useful for treating T-ALL patients.

Although combinatorial chemotherapeutic approaches increase the overall survival, in particular young patients suffer from long-term side effects. Besides the side effects, relapsed T-ALL patients mostly display resistance to chemotherapy. Therefore,

* Corresponding author. Division of Translational Cancer Research, Department of Laboratory Medicine, Lund University, Medicon Village 404:C3 Scheelevägen 8, 22363 Lund, Sweden.
E-mail address: kazi.uddin@med.lu.se (J.U. Kazi).

http://dx.doi.org/10.1016/j.canlet.2017.07.019
0304-3835/

development of novel targeted therapies is needed to avoid long-term side effects. Since NOTCH1 is the major oncogene in T-ALL, targeting NOTCH1 using monoclonal antibodies or inhibitors against NOTCH1 activating proteins (such as gamma-secretase or ADAM10) displays *in vitro* and *in vivo* antitumor activity [10–12]. Furthermore, inhibitors targeting JAK, STAT and ABL1 can be useful in treating patients carrying activating mutations of IL7R, JAK1 or JAK3 and ABL1 [7]. PI3K, MEK1/2 and mTOR inhibitors are also being tried in clinical trials.

Activating mutations of NOTCH1 activates CDKs. NOTCH1 activation results in expression of cyclin D3 through direct modulation of promoter activity [13]. Furthermore, activation of NOTCH signaling induces RB phosphorylation as well as CDK4 and CDK6 expression in primary T cells [13]. NOTCH1 intracellular domain binds to the promoter region of the SKP2 gene that encodes an E3 ubiquitin ligase. Aberrant expression of SKP2 negatively regulates cyclin-dependent kinase inhibitor CDKN1B expression and subsequently accelerates cell cycle progression [14]. Therefore, it is likely that T-ALL cells have accelerated cell cycle regulation due to different genetic changes. A recent study suggests that CDK4/6 inhibitors display synergy with chemotherapies in preclinical settings [15]. In this report, using a panel of T-ALL cell lines and a panel of kinase inhibitors, we identified CDK inhibitor dinaciclib as a potential drug for T-ALL. We show that dinaciclib potently inhibits the growth of T-ALL cells, induces apoptosis and extends survival of T-ALL xenograft mice.

Materials and methods

Reagents and cell lines

The human T-ALL cell lines, PF-382, P12-ICHIKAWA, CTV-1, CML-T1, LOUCY, DND-41, KE-37, ALL-SIL, SUP-T1, MOLT3, CCRF-CEM and Jurkat were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) and maintained in RPMI 1640 supplemented with 10%–20% heat-inactivated fetal bovine serum (Gibco, Thermo Scientific). Anti-c-MYC, anti-survivin, anti-cyclin T1, anti-cleaved PARP, and anti-beta-Actin antibodies were from Santa Cruz Biotechnology.

Cell viability assay

Cells were seeded in 96-well plates in the presence or absence of different concentrations of inhibitor. PrestoBlue (Thermo Scientific) was added (1:10) to cells after 46 h of inhibitor treatment, and further incubated for 2 h. Fluorescence was measured using a multi-well plate reader.

Kinase inhibitor library

A kinase inhibitor library including 378 kinase inhibitors was obtained from Selleck Chemicals. The concentrated inhibitors (10 mM in DMSO) were diluted to 100 nM and 1000 nM using cell culture medium. Cell viability assays using PrestoBlue (Thermo Scientific) were used to examine the effect of inhibitors.

Apoptosis assay

Cells were seeded in 12-well plates in the presence or absence of different concentrations of inhibitor. Two days after seeding, cells were analyzed using Annexin V-APC and 7-AAD Kit (BD Biosciences). Cells positive for either Annexin V alone or Annexin V and 7AAD were counted as apoptotic cells.

Colony formation assay

One thousand cells were cultured in 80% human methylcellulose medium in the presence or absence of inhibitor in a 24-well plate as described earlier [16,17]. Cells were grown for seven days and colonies were counted by two independent researchers.

Cell cycle analysis

Cells were treated with inhibitor overnight before fixing with prechilled ethanol. Cells were then stained with FxCycle™ Violet stain (Thermo Fisher Scientific) and analyzed by flow cytometry.

Xenograft study

Ten NSG mice were injected with 2.5 million CCRF-CEM cells through the tail vein [18]. Four days after injection of cells, five mice were treated with 36 mg/kg dinaciclib (intravenous through the tail vein), and five mice were treated with vehicle (20% 2-hydroxypropyl beta-cyclodextrin in water) for five days a week with two days off treatment. Dinaciclib treatment continued before euthanization. The experiment was performed with the ethical permit obtained from the Swedish Animal Welfare Authority following approved guidelines.

Results

T-ALL cell lines CCRF-CEM and Jurkat display high sensitivity to CDK inhibitors

To understand the possible involvement of kinases in T-ALL pathogenesis, we used a panel of kinase inhibitors including 378 inhibitors against different kinases. We observed that several inhibitors targeting the PI3K/AKT/mTOR pathway and cell cycle displayed growth inhibitory effect at 100 nM (Fig. 1A) and 1000 nM (Fig. 1B) concentrations. Furthermore, a closer look at the data indicated that inhibitors against CDK, PLK, SRC PI3K/DNA-PK and PI3K/HDAC displayed equally good inhibitory effect, while inhibition of Aurora kinases resulted in selective growth inhibition in CCRF-CEM cells (Fig. 1C and D). Loss of FBXW7 and TP53 function results in constitutive activation of Aurora kinase activity. Sequencing data suggest the presence of loss-of-function mutations in the FBXW7 and TP53 genes in both the cell lines (data not shown). However, it is unclear why CCRF-CEM displays higher selectivity to Aurora kinase inhibitors. Interestingly we observed that the CDK inhibitor dinaciclib displayed equal growth inhibition of both the cell lines. Therefore, we decided to continue with dinaciclib for further experiments.

The CDK inhibitor dinaciclib displays significant growth inhibition in a panel of cell lines

The CDK inhibitor dinaciclib recently completed a Phase III trial for patients with refractory chronic lymphocytic leukemia (NCT01580228). Therefore, it would be highly beneficial for patients if it could be used for T-ALL. We used 0 nM, 10 nM, 50 nM and 100 nM concentrations of dinaciclib and observed that 50 nM concentration significantly reduced cell viability of both CCRF-CEM and Jurkat cells after 48 h (data not shown) incubation. To understand whether the drug is effective for a wider range of cell lines, we used a panel of 12 T-ALL cell lines with 50 nM dinaciclib concentration. We observed that cells are highly sensitive to dinaciclib thereby displaying a significant reduction of cell viability (Fig. 2A). Thus, we suggest that dinaciclib is an effective drug for a wider range of T-ALL cells.

Dinaciclib inhibits expression of c-MYC and cyclin T1, and induces G2/M phase cell cycle arrest

To understand how dinaciclib acts on the molecular level, we analyzed expression of cyclin T1 and c-MYC. Cells treated with 50 nM or higher concentration of dinaciclib showed reduced level of cyclin T1 expression (Fig. 3A). Furthermore, 10 nM dinaciclib was effective to reduce the c-MYC expression, while 50 nM concentration completely abolished the c-MYC expression (Fig. 3B). Additionally, we analyzed cell cycle in the presence of increasing concentrations of dinaciclib. We observed that dinaciclib treatment did not alter the cell population in G0/G1 phase. The cell population in S-phase was significantly decreased while the population in G2/M phase was significantly increased (Fig. 3C). Therefore, it is