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Research paper

Increased activity of both CDK1 and CDK2 is necessary for the combinatorial activity of WEE1 inhibition and cytarabine

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ABSTRACT

Inhibition of WEE1 is emerging as a promising chemosensitization strategy in many cancers including acute leukemia. Our lab and others have demonstrated that a small-molecule inhibitor of WEE1, AZD1775, sensitizes acute leukemia cells to cytarabine; however, a mechanism of combinatorial activity has remained elusive. Thus, we sought to determine the relative contribution of WEE1 targets CDK1 and CDK2 to the combinatorial activity of AZD1775 and cytarabine. To accomplish this, we expressed "WEE1 resistant" CDK1 (CDK1-AF) and CDK2 (CDK2-AF) constructs in a T-ALL cell line. Expression of CDK1/2-AF together, but neither alone, enhanced the anti-proliferative effects, DNA damage and apoptosis induced by cytarabine. Furthermore, pharmacologic inhibition of CDK1 alone or CDK1 and CDK2 together reduced the combinatorial activity of AZD1775 and cytarabine. Thus, increased activity of both CDK1 and CDK2 in response to WEE1 inhibition is necessary for the combinatorial activity of AZD1775 and cytarabine. This suggests the role of WEE1 in cells with accumulated DNA damage extends beyond regulation of CDK1 and the G2/M checkpoint and highlights the importance of WEE1 in mediating progression through the cell cycle.

1. Introduction

Advances such as risk-directed chemotherapy have substantially improved survival in AML and ALL [7]. However, novel therapies are needed for patients who relapse or cannot tolerate standard induction treatment. One strategy to improve outcomes is addition of targeted agents that enhance the efficacy of chemotherapies currently used to treat these diseases. WEE1 is emerging as a promising target in cells treated with genotoxic chemotherapies. WEE1 is a tyrosine kinase that inhibits CDK1 and CDK2 by phosphorylation at tyrosine 15. In response to DNA damage, WEE1 activity is upregulated to promote cell cycle arrest at the intra-S or G2/M checkpoint [17,19]. This promotes DNA damage repair prior to entry into mitosis and allows cells to avoid DNA damage-induced apoptosis or propagation of mutations to daughter cells.

Our lab previously demonstrated that AZD1775, a small-molecule inhibitor of WEE1, enhances the anti-proliferative effects of cytarabine (Ara-C) in AML and T-ALL cells by abrogating the S-phase arrest induced by Ara-C and increasing DNA damage [6,21]. This work, along with the work of others, provided support for a clinical trial examining the efficacy of AZD1775 combined with Ara-C in AML (NCT02666950). As this combination progresses to clinic trials, a complete understanding the mechanism of the combinatorial activity of AZD1775 and Ara-C will be necessary to identify patients most likely to benefit from treatment. While a number of studies have examined alterations in the cell cycle in response to AZD1775 and anti-metabolite chemotherapies such as Ara-C, the relative contribution of WEE1 targets CDK1 and CDK2 has not been studied. Thus, we sought to understand the contribution of CDK1 and CDK2 to the combinatorial activity of AZD1775 and Ara-C. We hypothesized that increased activity of CDK1 and/or CDK2 is required for the combinatorial activity of AZD1775 and Ara-C. Using genetic models of "WEE1 resistant" CDK1 and CDK2, we demonstrate that increased activity of both CDK1 and CDK2 is required to enhance the anti-proliferative effects of Ara-C in a T-ALL cell line. This is confirmed by pharmacologic data demonstrating inhibition of CDK1 alone or CDK1 and CDK2 together can reduce the combinatorial effect of AZD1775 and Ara-C. Together, these data highlight the unique ability of WEE1 to regulate two CDKs that function in different phases of the cell cycle.

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2. Materials and methods

2.1. Cell culture

Jurkat and MV4;11 cells were generous gifts from the laboratories of Drs. Douglas Graham and James DeGregori. Cell lines were DNA fingerprinted by multiplex PCR using the Profiler Plus or Identifier Kits (ABI) as previously described [23], and periodically tested for Mycoplasma by PCR. Cells were cultured in RPMI with 10% FBS and penicillin/streptomycin at 37 °C in humidified air supplemented with 5% CO_2 and maintained in culture for no longer than 2 months.

2.2. Lentivirus preparation

CDK1/2-AF constructs were generously provided by Dr. David O. Morgan and cloned into response vectors of the Lenti-X Tet-On 3G Inducible System (Clontech Laboratories, Inc.) Virus-containing media was prepared according to manufacturer's protocol.

2.3. Chemicals, antibodies, and reagents

AZD1775 was provided by AstraZeneca (Wilmington, DE). Cytarabine, doxycycline, and puromycin were purchased from Sigma-Aldrich and diluted in water. RO-3306 and roscovitine were purchased from EMD Millipore (Billerica, MA) and diluted in DMSO. Antibodies against HA-tag, GAPDH, PARP, γH2AX, tubulin, and actin were purchased from Cell Signaling Technology (Danvers, MA).

2.4. Flow cytometry

Cell viability was determined with the Guava EasyCytePlus (Millipore, Billerica, MA) by measuring cell counts with propdium iodide exclusion.

2.5. Statistical analysis

Data analysis and graphing was performed using Graphpad Prism 5 (GraphPad Software, La Jolla, CA). Unless otherwise indicated, graphs represent the mean from a minimum of three biological replicate experiments, and error bars portray the standard error of the mean. One-way ANOVA was used to compare 3 or more samples with a single variable. The Bonferroni correction was applied to determine significance between any two conditions. Non-linear regression was used to generate dose-response curves and determine IC50 values.

3. Results

3.1. Expression of WEE1-resistant CDK1 and CDK2 enhances the anti-proliferative effects of cytarabine

Inhibition of WEE1 reduces inhibitory phosphorylation of CDK1 and CDK2 in response to DNA damage. This prevents cell cycle arrest at the intra-S or G2/M checkpoint and results in an increase in apoptosis [9,21]. However, it is not known whether increased activity of one or both CDKs is required to enhance the efficacy of DNA damaging agents. We sought to determine the necessity of CDK1/2 activity in the context of Ara-C treatment by expressing 'WEE1-resistant' CDK constructs in the T-ALL cell line Jurkat. These mutant CDK constructs substitute alanine for threonine at position 14 and phenylalanine for tyrosine at position 15 yielding CDK constructs that cannot be inhibited by phosphorylation (CDK1/2-AF). Previous reports have confirmed expression of these constructs results in increased CDK activity [11]. The CDK1/2-AF constructs were cloned into doxycycline-inducible vectors, and expression of the HA-tagged proteins was observed when cells were treated with doxycycline (Fig. 1a). To test the effects of CDK1/2-AF expression on cell viability in cytarabine-treated cells, Jurkat cells transduced with vector control, CDK1-AF, and/or CDK2-AF plasmids were treated with Ara-C with and without doxycycline. Expression of CDK1-AF or CDK2-AF alone did not sensitize cells to cytarabine, but we observed significant sensitization when both CDK1-AF and CDK2-AF were expressed (Fig. 1b). Next, these cell lines were treated with Ara-C in concentrations ranging from 0.5 nM to 40 nM. Doxycycline-induced expression of CDK1-AF or CDK2-AF alone did not shift the dose-response curve of Ara-C. However, a shift of the dose-response curve was observed in cells expressing both CDK1-AF and CDK2-AF. The IC50 value of Ara-C was 6.09 nM when expression of the CDK constructs was not induced, and this decreased to 2.86 nM when both constructs were expressed (Fig. 1c). Thus, increased activity of both CDK1 and CDK2 enhances the anti-proliferative effects of Ara-C.

3.2. Expression of WEE1-resistant CDK1 and CDK2 increases DNA damage and apoptosis in cytarabine-treated cells

Our lab and others have demonstrated that AZD1775 enhances DNA damage induction and apoptosis in cells treated with Ara-C [6,21,22]. Therefore, we questioned whether increased activity of CDK1 and CDK2 was responsible for these events. Jurkat cells expressing CDK1-AF and/or CDK2-AF were treated with Ara-C and/or doxycycline as well as Ara-C and AZD1775 as a positive control. Expression of CDK1-AF or CDK2-AF alone did not increase apoptosis as evidenced by a lack of cleaved PARP. However, expression of both constructs produced cleaved PARP comparable to levels observed in cells treated with Ara-C and AZD1775 (Fig. 2). We observed a slight increase in γH2AX in cells expressing either CDK1-AF or CDK2-AF suggesting that increased activity of either CDK can induce some DNA damage in cells exposed to Ara-C. However, more DNA damage occurred when activity of both CDK1 and CDK2 increased. Surprisingly, expression of both CDK constructs induced less DNA damage than AZD1775 in cytarabine-treated cells (Fig. 2). Thus, increased activity of both CDK1 and CDK2 increases DNA damage and apoptosis, although DNA damage induction is greater in cells treated with AZD1775.

3.3. Pharmacologic inhibition of CDK1 and CDK2 reduces the combinatorial activity of AZD1775 and cytarabine

We then asked whether pharmacologic inhibition of CDK1 alone or both CDK1 and CDK2 could reduce the combinatorial activity of AZD1775 and Ara-C. RO-3306 is an ATP-competitive inhibitor of CDK1. The drug is 10-fold more selective for CDK1 than for CDK2 and 50-fold more selective for CDK1 than for CDK4 and CDK6. [24]. AZD1775 treatment significantly enhanced the anti-proliferative effect of Ara-C in Jurkat cells, but the effect of the combination was reduced when cells were treated with RO-3306 (Fig. 3a). This is consistent with the findings in CDK1/2-AF-expressing Jurkat cells which suggests increased activity of both CDK1 and CDK2 is necessary for the combinatorial activity of AZD1775 and Ara-C. Next, cells were treated with roscovitine, an ATP-competitive inhibitor of CDK1, CDK2, CDK5, and CDK7 [3]. Inhibition of CDK1 and CDK2 with roscovitine reduced the combinatorial effect of AZD1775 and Ara-C in Jurkat cells as well as in the AML cell line, MV;411 (Fig. 3b,c). Roscovitine treatment also reduced apoptosis as evidenced by reduced cleaved PARP in cells treated with AZD1775 and Ara-C (Fig. 3d). Together, these data confirm increased activity of CDK1 and CDK2 is required for the combinatorial activity of AZD1775 and Ara-C.

4. Discussion

Inhibition of WEE1 sensitizes cancer cells to many DNA damaging agents; however, the mechanisms of sensitization are not fully understood. Our group previously reported that AZD1775 sensitized AML and T-ALL cells to Ara-C by abrogating S-phase arrest and promoting apoptosis over DNA damage repair [6,21]. This work, combined with