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Intracellular cytarabine triphosphate production correlates to deoxycytidine kinase/cytosolic 5′-nucleotidase II expression ratio in primary acute myeloid leukemia cells

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ABSTRACT

Cytarabine (ara-C) is the key agent for treating acute myeloid leukemia (AML). After being transported into leukemic cells by human equilibrative nucleoside transporter 1 (hENT1), ara-C is phosphorylated to ara-C triphosphate (ara-CTP), an active metabolite, and then incorporated into DNA, thereby inhibiting DNA synthesis. Deoxycytidine kinase (dCK) and cytosolic 5′-nucleotidase II (cN-II) are associated with the production of ara-CTP. Because ara-C's cytotoxicity depends on ara-CTP production, parameters that are most related to ara-CTP formation would predict ara-C sensitivity and the clinical outcome of ara-C therapy. The present study focused on finding any correlation between the capacity to produce ara-CTP and ara-C-metabolizing factors. In vitro ara-CTP production, mRNA levels of hENT1, dCK, and cN-II, and ara-C sensitivity were evaluated in 34 blast samples from 33 leukemic patients including 26 with AML. A large degree of heterogeneity was seen in the capacity to produce ara-CTP and in mRNA levels of hENT1, dCK, and cN-II. Despite the lack of any association between each of the transcript levels and ara-CTP production, the ratio of dCK/cN-II transcript levels correlated significantly with the amount of ara-CTP among AML samples. The HL-60 cultured leukemia cell line and its three ara-C-resistant variants (HL-60/R1, HL-60/R2, HL-60/R3), which were 8-, 10-, and 500-fold more resistant than HL-60, respectively, were evaluated similarly. The dCK/cN-II ratio was again proportional to ara-CTP production and to ara-C sensitivity. The dCK/cN-II ratio may thus predict the capacity for ara-CTP production and ultimately, ara-C sensitivity in AML.

1. Introduction

Cytarabine, or 1-β-D-arabinofuranosylcytosine (ara-C), is the key agent for treating acute myeloid leukemia (AML). The combination of regular-dose ara-C given for 7 days with daunorubicin given for 3 days has been a standard induction therapy for AML. This regimen achieves complete remission rates of ≥70% in adult AML patients [1,2]. However, these remissions are not durable, and long-term survivors account for only 30–40%. To improve clinical outcomes, individualization of treatment is needed according to prognostic factors including chemosensitivity in each patient. In this regard, the sensitivity of leukemic cells to ara-C may be crucial for predicting therapeutic efficacy and establishing tailor-made chemotherapy.

As the mechanism of action, ara-C is transported into leukemic cells by membrane transporters including the human equilibrative nucleoside transporter 1 (hENT1) [3]. Inside the cell, ara-C is phosphorylated by the rate-limiting enzyme deoxycytidine kinase (dCK) to ara-C monophosphate, and then to ara-C diphosphate by deoxycytidine monophosphate kinase and eventually to ara-C triphosphate (ara-CTP), an active metabolite of ara-C, by nucleoside diphosphate kinase [4]. Ara-C catabolism results from rapid deamination by cytidine deaminase to the non-toxic metabolite ara-U [4], while cytosolic 5′-nucleotidase II (cN-II) dephosphorylates ara-CMP [5], thereby preventing production of the active form. Ara-CTP is then incorporated into DNA strands during the S phase of the cell cycle, thereby inhibiting DNA synthesis [6–8]. As drug incorporation into DNA is the product of the ara-CTP concentration and time, ara-CTP levels represent an index of ara-C cytotoxicity. Furthermore, the clinical value of intracellular ara-CTP level has been established with the identification of a

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Abbreviations: ara-C, cytarabine or 1-β-D-arabinofuranosylcytosine; ara-CTP, cytarabine triphosphate or 1-β-D-arabinofuranosylcytosine triphosphate; AML, acute myeloid leukemia; hENT1, human equilibrative nucleoside transporter 1; IC_{50}, 50% growth-inhibitory concentration; dCK, deoxycytidine kinase; cN-II, cytosolic 5′-nucleotidase II; XTT, sodium 3′-(1-[(phenylamino)-carbonyl-3,4-tetrazolium])-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate.

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correlation between the intracellular pharmacokinetics of ara-CTP and response to ara-C therapy [9–13].

If the capacity of patients' leukemic cells to produce ara-CTP is determined prior to therapy, sensitivity to ara-C-based chemotherapy and ultimately the clinical outcome could be predicted. As the production of ara-CTP depends on the cellular function of ara-C metabolism in each patient's leukemia, previous studies sought to identify any relationship between clinical outcomes of ara-C-based chemotherapy and ara-C-related factors including hENT1, dCK, and cN-II [14–18]. However, results were inconsistent, since the studies dealt with patients having different genetic backgrounds [14–18]. More importantly, no exact correlation was revealed between the capacity to produce ara-CTP and ara-C-metabolizing factors in leukemic cells. The factors that are functionally most crucial to the production of ara-CTP thus remain undetermined.

We hypothesized that factors that can correlate with ara-CTP production may offer a predictor for ara-C sensitivity and its therapeutic efficacy. The present study focused on finding any correlation between the capacity to produce ara-CTP and ara-C-metabolizing factors in patients' leukemic cells. For this purpose, we quantitated the in vitro production of intracellular ara-CTP, mRNA expression levels of hENT1, dCK, and cN-II, and ara-C sensitivity in blasts from leukemic patients including those with AML. In addition, we evaluated four cultured leukemia cell lines with different sensitivities to ara-C in parallel experiments.

2. Materials and methods

2.1. Patient samples

The present study was approved by the ethics committee of the University of Fukui Hospital, and informed consent was obtained from each patient. A total of 34 leukemic cell samples from 33 leukemic patients were evaluated (Table 1). One patient was evaluated at diagnosis and again at relapse after the first remission following induction chemotherapy. The evaluated leukemia included 26 cases of AML, 4 cases of acute lymphoblastic leukemia, 2 cases of plasma cell leukemia, and 1 case of leukemic manifestation of peripheral T cell lymphoma (Table 1). The diagnosis of leukemia was made by standard cytological and histochemical examination of bone marrow smears according to the French–American–British criteria [19].

Table 1
Patient characteristics.

No. of patients evaluated	33
Sex (*n*)	
Male	17
Female	16
Age (years)	
Median	69
Range	18–86
Diagnosis	Number
ML-LM	1
ALL (L2)	3
CLL	1
PCL	2
AML (M0)	2
AML (M1)	7
AML (M2)	12
AML (M4)	2
AML (M5)	2
AML (M7)	1

No. or *n*, number; years, age; ML-LM, leukemic manifestation of malignant lymphoma; ALL, acute lymphoblastic leukemia; PCL, plasma cell leukemia; AML, acute myeloid leukemia; M0-7, subclass of FAB classification.

Prior to chemotherapy, peripheral blood was drawn into heparinized tubes, layered over Ficoll-Hipaque (Beckman-Couler Japan, Tokyo, Japan), and centrifuged ($500 \times g$, 30 min, room temperature) to isolate leukemic cells [9]. The cells were washed twice with phosphate-buffered saline and then centrifuged ($500 \times g$, 5 min, 4 °C) to pellet the cells. Aliquots were resuspended in RPMI1640 media supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a 5% CO_2-humidified atmosphere for further experiments.

2.2. Preparation of cultured leukemic cells

This investigation used human leukemia HL-60 cells and its three ara-C-resistant variants, HL-60/R1, HL-60/R2, and HL-60/R3, which had been established previously [20,21]. In brief, to develop ara-C-resistant variants, parental HL-60 cells were cultured independently in three flasks in media containing ara-C. The initial concentration of ara-C was half the 50% growth-inhibitory concentration (IC_{50}) for HL-60 cells. Cultures were observed daily and allowed to grow. Drug concentrations on subsequent passages were gradually increased, and one cell line resistant to ara-C was cloned from each flask using the limiting dilution method. The three independent ara-C-resistant HL-60 variants were named HL-60/R1, HL-60/R2, and HL-60/R3. Reduced dCK activity was demonstrated in R1 and R2 cells, while increased cN-II activity was also present in R2 cells [20,21]. R3 cells almost lacked dCK activity [20,21]. These cell lines were maintained in RPMI1640 media supplemented with 10% heat-inactivated fetal bovine serum at 37 °C in a 5% CO_2-humidified atmosphere.

2.3. Determination of intracellular ara-CTP

HPLC analysis was used to determine intracellular ara-CTP production in vitro [9]. Briefly, both primary and cultured leukemic cells (1×10^6 ml^{-1}, 10 ml) were incubated with different concentrations of ara-C for 6 h. Cells were collected by centrifugation ($500 \times g$, 5 min, 4 °C), followed by extraction of the acid-soluble fraction, the nucleotide pool. The acid-soluble fraction was then applied to the HPLC procedure using a TSK gel DEAE-2 SW column (length, 250 mm; internal diameter, 4.6 mm, TOSOH, Tokyo, Japan) and 0.06 M Na_2HPO_4 (pH 6.9)—20% acetonitrile buffer. The ara-CTP peak was identified solely by its retention time and quantitated by its peak area at an absorbance of 269 nm. The ara-CTP concentration was expressed as pmol/10^7 cells.

2.4. Determination of transcript levels of dCK, cNT-II, and hENT1

To evaluate mRNA levels of hENT1 (accession: NM_001078177), dCK (accession: NM_000788), and cN-II (accession: NM_012229), real-time RT-PCR was performed using the ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, CA, USA). Briefly, total RNA was isolated from each sample (1×10^7 cells) using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and subjected to reverse transcription to prepare cDNA using SuperScript-RNase H-Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). Quantitation of the target cDNA and an internal reference gene (GAPDH) was then conducted using TaqMan Gene Expression Assays (Applied Biosystems). The PCRs were performed using TaqMan universal PCR Master Mix according to the manufacturer's instructions. Primers for hENT1, dCK, and cN-II were purchased from Applied Biosystems. The relative standard curve quantitation method was used. The values for cultured human leukemia K562 cells were set as controls, and values of given samples were determined as the ratio compared with the value of K562 cells. We used K562, because its transcript levels were closer to those in patients' leukemic cells than those in HL-60 were.

2.5. Proliferation assay

To evaluate the growth inhibition effect of ara-C on primary leukemic cells, the trypan blue dye exclusion assay was performed [21]. In brief, 1 ml of cells (1×10^5 ml^{-1}) was incubated for 72 h with different concentrations of ara-C. The cells then were mixed with an equal amount of Trypan blue solution. Viable cells were counted under light microscopy. For cultured leukemic cells, 1 ml of cells (5×10^4 ml^{-1}) was pre-incubated for 24 h, followed by the addition of a 10-μl aliquot of different concentrations of ara-C. Cells were incubated for a further 72 h, and applied to the sodium 3′-(1-[(phenylamino)-carbonyl-3,4-tetrazolium])-bis(4-methoxy-6-nitro)benzene sulfonic acid hydrate (XTT) assay according to the manufacturer's instructions (Roche, Indianapolis, IN, USA) with slight modifications [21]. The IC_{50} value was extrapolated from a growth inhibition curve.

2.6. Statistical analyses

All statistical analyses were performed using Microsoft Excel 2007 software (Microsoft, Redmond, WA, USA). All graphs, linear regression lines, and curves were generated using GraphPad Prism software (version 5.0) (GraphPad Software, Inc. San Diego, CA, USA). Values of $P \leq 0.05$ were considered statistically significant.

3. Results

3.1. Intracellular ara-CTP production and transcript levels of hENT1, dCK, and cN-II, in primary leukemic cells

Intracellular ara-CTP production was evaluated in vitro for 33 of 34 primary leukemic cell samples. When cells were incubated with ara-C, ara-CTP production increased according to the ara-C concentration in media. Incubation with 10 μM ara-C for 6 h produced a mean ara-CTP concentration of 1047 pmol/10^7 cells, with a range of 86.7–3135 pmol/10^7 cells, indicating wide variability among samples (Fig. 1). No specific tendencies were observed regarding subtypes of leukemia. These results thus suggested different capabilities for yielding ara-CTP among patient samples.

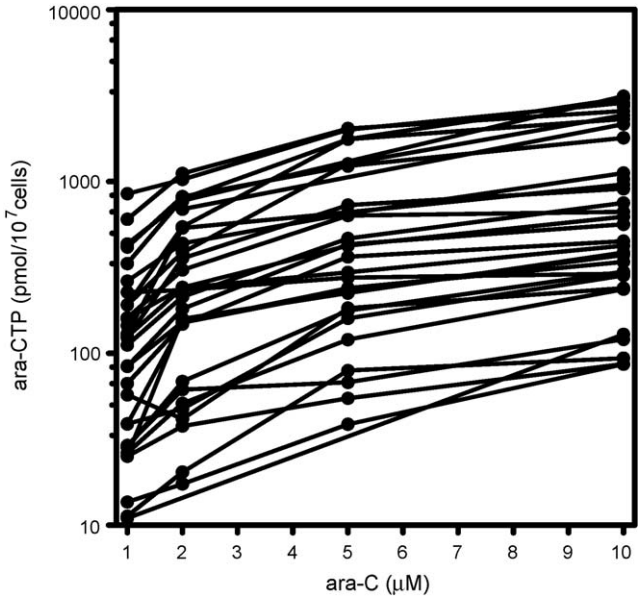

Fig. 1. In vitro ara-CTP production in 33 primary leukemic cells. Primary leukemic cells (1×10^6 cell/ml, 10 ml) were incubated for 6 h with various concentrations (1, 2, 5, 10 μM) of ara-C in media, followed by extraction of the nucleotide pool and measurement of intracellular ara-CTP concentrations using HPLC.

Transcript levels of ara-C-related factors were determined in samples from 30 patients. Median levels of hENT1, dCK, and cN-II were 3.39 (range, 0.01–22.16), 16.41 (range, 0.09–195.00), and 36.947 (range, 0.26–302.00), respectively. Again, large heterogeneity was apparent in the level of each factor among samples, and no specific tendencies according to the specific pathology were seen. These results thus suggested that the different abilities to yield ara-CTP might be attributable to wide variations in ara-C-related factors.

3.2. Relationship between the transcript levels of ara-C-related factors and the intracellular ara-CTP production

Ara-C-related factors were sought for any correlation to intracellular ara-CTP production using 27 complete sets from all patients' samples. Disappointingly, intracellular ara-CTP concentrations did not correlate with any of the factors among samples (Fig. 2A, C, and E). No correlation was found even by focusing on 20 complete sets from AML samples (Fig. 2B, D, and F). However, if the dCK/cN-II ratio to represent the production/degradation ratio, a significant correlation was obtained between this ratio and ara-CTP production among AML samples (Fig. 2H), but not among all samples (Fig. 2G). These results thus suggested that the ability to produce ara-CTP depends on the dCK/cN-II ratio in primary AML cells.

3.3. Cytotoxicity of ara-C in primary leukemic cells

To demonstrate the importance of ara-CTP in the cytotoxicity of ara-C, 8 AML samples were incubated with different concentrations of ara-C, followed by evaluation of cellular viability after 72 h. Sensitivity to ara-C varied widely among samples, with the IC_{50} ranging from 0.26 μM to 360 μM (Fig. 3A). IC_{50} value appeared inversely correlated to the ara-CTP concentration, i.e., a lower IC_{50} was associated with greater ara-CTP production (Fig. 3B), suggesting that the intracellular ara-CTP is a crucial metabolite for ara-C sensitivity. This correlation was not strong enough to be significant, probably because ara-C is a cell-cycle specific agent, and primary cells were unlikely to proliferate vigorously in media for days.

Among the above 20 AML patients, 12 received chemotherapy while 8 were not candidates for intensive treatment because of their poor general conditions or old ages. Although the 12 patients received different types of chemotherapies according to the setting (de novo or relapsed), the dCK/cN-II ratio was examined to have any correlation to therapeutic outcomes. Levels of the ratio appeared to be higher in responders than in nonresponders (Fig. 3C). The difference was not significant ($P > 0.05$) and treatment schedules were not uniform, however, the ratio was suggested to be a predictor of therapeutic efficacy.

3.4. Changes in ara-C-related factors and intracellular ara-CTP productions after relapse in an AML patient

One AML patient was evaluated at first onset and at the disease relapse. The patient received induction chemotherapy using ara-C and idarubicin, achieving complete remission. Despite three cycles of consolidation therapy using an ara-C-based regimen, the disease relapsed 4 months later. Salvage therapy was unsuccessful, and the patient died of pneumonia. The in vitro ara-CTP production at relapse (109 pmol/10^7 cells) was apparently lower than at onset (335 pmol/10^7 cells) (Fig. 4A). Transcript levels of dCK were reduced, while levels of cN-II were increased at relapse (Fig. 4B and C). The dCK/cN-II ratio at relapse (0.218) was lower than at onset (0.425) (Fig. 4D), suggesting that the capacity to yield ara-CTP in cells was altered due to changes in ara-C-metabolizing factors during therapy.

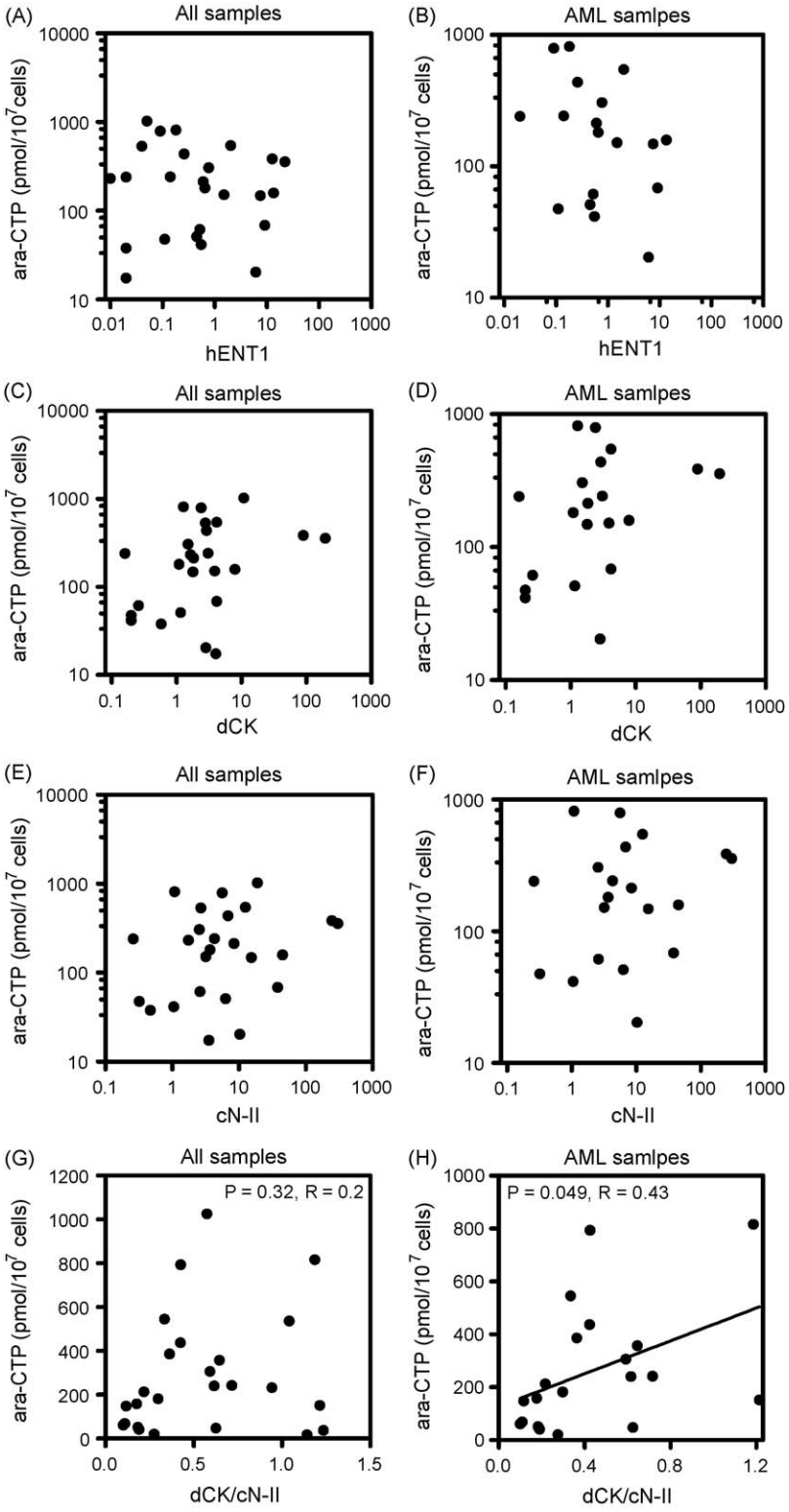

Fig. 2. The relationship between each ara-C-related factor and the intracellular ara-CTP production. (A) hENT transcript level and ara-CTP; (C) dCK transcript level and ara-CTP; (E) cN-II transcript level and ara-CTP; and (G) ratio of dCK/cN-II and ara-CTP, in 27 complete sets from all patients. (B) hENT transcript level and ara-CTP; (D) dCK transcript level and ara-CTP; (F) cN-II transcript level and ara-CTP; and (H) ratio of dCK/cN-II and ara-CTP, in 20 complete sets from AML patients. Intracellular ara-CTP levels were determined in primary leukemic cells that had been incubated in vitro with 2 μM ara-C for 6 h.

3.5. Ara-C-related factors and intracellular ara-CTP concentrations in a cultured leukemic HL-60 cell line and its ara-C-resistant variants

To confirm the present findings obtained from primary leukemic cells, similar investigations were performed using human leukemia cell line HL-60 and its three ara-C-resistant variants, HL-60/R1, HL-60/R2, and HL-60/R3, which had been developed in previous studies [20,21]. As determined using the XTT assay, HL-60/R1 and HL-60/R2 cells were moderately ara-C-resistant, while HL-60/R3 cells were much more highly resistant (Table 2). When these cell lines were

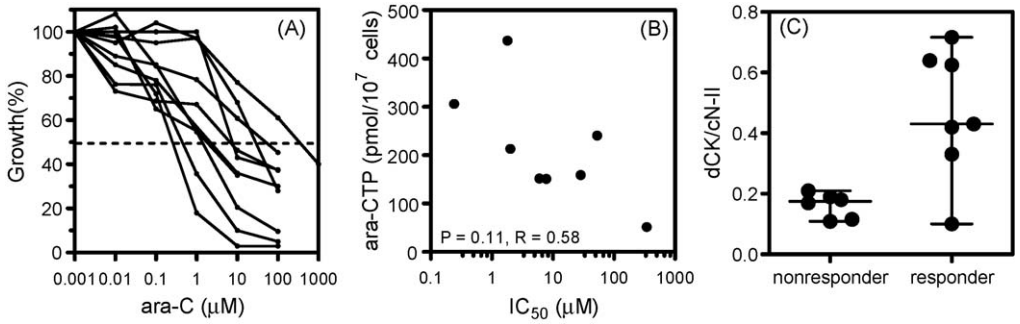

Fig. 3. (A) Viability of ara-C-treated primary leukemic cells. Leukemic cells from eight AML patients were incubated with various concentrations of ara-C for 72 h in vitro, followed by evaluation of cell viability using the Trypan blue dye exclusion assay. (B) Relationship between ara-CTP production and IC_{50} values in eight primary leukemic calls. Intracellular ara-CTP concentrations were determined when cells were incubated with 2 μM ara-C for 6 h. (C) Among 20 AML patients with complete data sets, 12 received chemotherapy, and the dCK/cN-II ratio was examined to have any correlation to therapeutic outcomes. Responders were patients who reached remission and nonresponders were patients refractory to treatment. The Bars represented median and range. The difference of two groups was statistically examined using the Mann–Whitney test.

incubated with ara-C, production of ara-CTP was reduced in the ara-C-resistant variants compared with the parental HL-60. These reductions in ara-CTP concentrations appeared to be correlated to the extent of drug resistance, indicating a critical role of ara-CTP in ara-C-induced cytotoxicity (Table 3). Transcript levels of dCK and cN-II were 0.1 and 0.12 for R1 and 0.1 and 0.14 for R2, respectively. These values might be associated with their kinase activities (reduced dCK in R1 and R2, increased cN-II in R2) [21]. Both R1 and R2 had a reduced ara-CTP, compared with the parental HL-60, but the reduction was slightly severe in R2 that had increased cN-II expression and activity. More importantly, dCK/cN-II ratio was again significantly correlated to ara-CTP concentrations (Fig. 5A) and, here, to cellular sensitivity to ara-C (Fig. 5B). These results suggested that dCK/cN-II ratio offers a predictor of ara-CTP production and, ultimately, ara-C sensitivity. These results obtained from experiments using cultured cell lines were thus consistent with those from primary leukemic cells from patients.

4. Discussion

The efficient formation of intracellular ara-CTP is indispensable for obtaining cytotoxic response after ara-C treatment [4,6–8]. The present study was conducted to identify parameters that would predict the capability to produce ara-CTP in patients' leukemic cells, as such parameters might determine cellular sensitivity to ara-C and thus clinical outcomes of ara-C-containing chemotherapy. A large degree of heterogeneity was apparent in the capacity for ara-CTP production among samples (Fig. 1), suggesting variability in drug sensitivity. This might be attributable to different expression levels of ara-C-related factors in samples. Despite the lack of any association between each of these factors and ara-CTP concentration, the ratio of dCK/cN-II expression levels was significantly correlated to ara-CTP production among AML patient samples (Fig. 2). This correlation was confirmed in experiments using ara-C-sensitive and ara-C-resistant cultured

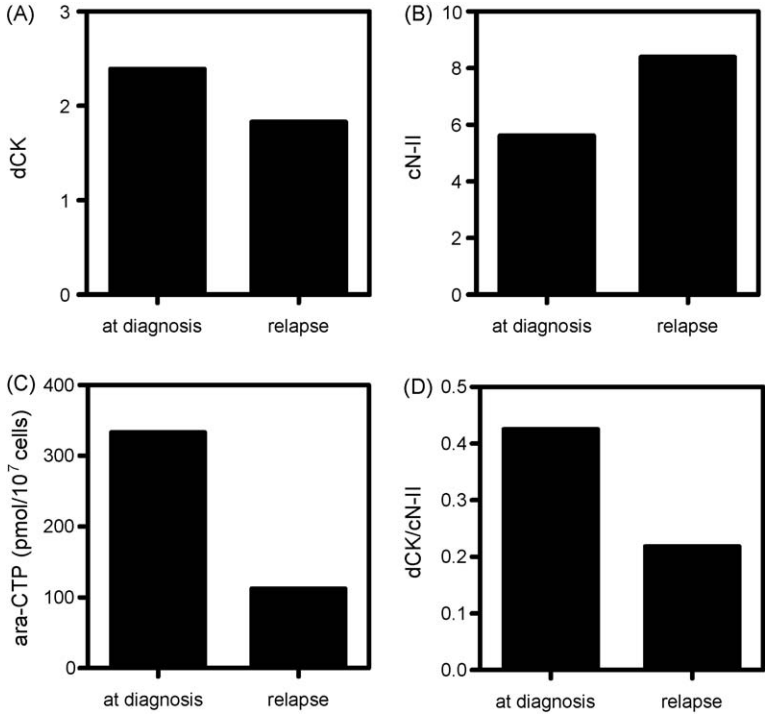

Fig. 4. Changes in transcript levels of ara-C-related factors and intracellular ara-CTP production within the same AML patient. Leukemic cells were obtained at diagnosis and at relapse after the first complete remission following induction chemotherapy and consolidation therapy. Intracellular ara-CTP concentration was determined when cells were treated with 2 μM ara-C for 6 h.

Table 2
Drug sensitivities of HL-60 and its ara-C-resistant variants (HL-60/R1, HL-60/R2, HL-60/R3) to ara-C.

Drugs	IC_{50} (μM)			
	HL-60	HL-60/R1	HL-60/R2	HL-60/R3
Ara-C	0.4	3.2	4.0	200.0
Relative resistance	–	8.0	10.0	500.0

Relative resistance was obtained by dividing the IC_{50} value of each ara-C-resistant cell line by that of the parental HL-60 cell line.

Table 3
Ara-CTP production of HL-60 and R1-3 cells.

Cell line	Ara-CTP (pmol/10^7cells)		
	Ara-C (2 μM)	Ara-C (5 μM)	Ara-C (10 μM)
HL-60	92 ± 60	394 ± 161	830 ± 401
HL-60/R1	72 ± 16	234 ± 79	322 ± 48
HL-60/R2	51 ± 32	176 ± 161	321 ± 177
HL-60/R3	0.3 ± 0.4	2.5 ± 2.6	36 ± 12

Ara-CTP was determined when cells were incubated with indicated concentrations of ara-C for 6 h.

leukemic cell lines (Fig. 5 and Tables 2 and 3). Reduced dCK/cN-II ratio appeared directly associated with ara-C resistance in primary cells (Figs. 3 and 4), which also was confirmed using cultured leukemic cells (Fig. 5).

Several studies have been performed to determine the relationship between ara-C-related factors and ara-C sensitivity for leukemic cells in vitro, as well as clinically. Using cultured leukemic cell lines, deficient dCK activity, decreased nucleoside transporter content, decreased DNA polymerase sensitivity, overexpression of cytidine deaminase gene, and enhanced cN-II activity have been associated with ara-C resistance in vitro [22–28]. Using leukemic cells from patients, Preisler et al. reported that ara-CTP retention correlated with duration of remission in 91 AML patients [29]. This suggested ara-CTP as the critical predictor for therapeutic outcomes of ara-C-based chemotherapy. However, direct measurement of intracellular ara-CTP on a larger scale may be somewhat difficult, given the technical complexity of the measurement. Subsequent studies then focused on determining ara-C-related factors in stored leukemic samples. Among these, the transcript level of hENT1 correlated with the in vitro sensitivity to ara-C for MLL gene-rearranged infant acute lymphoblastic leukemia or childhood AML cells [17,30]. The expression level of dCK determined in vitro ara-C sensitivity of blasts from children with Down syndrome and AML [31] or pediatric malignancies including leukemia [32]. Galmarini et al. demonstrated a close relationship between transcript levels of cN-II and disease-free survival of 108 AML patients [14]. They also showed that the cN-II/dCK ratio was a strong and independent prognostic factor in 115 AML patients treated with ara-C-containing regimens [16]. Suzuki et al. demonstrated in patients with high-risk myelodysplastic syndrome who received ara-C-containing chemotherapies that higher cN-II mRNA expression correlated to shorter median overall survival and shorter median post-chemotherapy survival [18]. Other ara-C-related parameters have not been found to be prognostic markers of ara-C-based chemotherapy [15]. Reports have thus been inconsistent, as each study has dealt with a different subtype of leukemia or a different therapeutic setting. Nevertheless, hENT1, dCK, and cN-II have been the only markers extracted as clinically significant, and were thus analyzed in the present study.

Both dCK and cN-II are involved in regulating the continuous and balanced supply of the four deoxyribonucleoside triphosphates. dCK is the enzyme in the first phosphorylation step of nucleoside monophosphate formation. cN-II is one of the seven 5′-nucleotidases that catalyze the dephosphorylation of ribo- and deoxyribonucleoside monophosphates, thereby counteracting the action of nucleoside kinases [33]. Both dCK and cN-II are also responsible for intracellular concentrations of the active metabolites of anti-cancer nucleoside analogs, including ara-C. The ratio between dCK and cN-II transcript levels in AML blasts has been shown to predict disease-free survival and overall survival [16]. The clinical efficacy of a similar nucleoside analog, cladribine, also depended on the ratio of dCK and cN-II expression levels in chronic lymphocytic leukemia [34]. However, whether the relationship between dCK/cN-II ratio and clinical outcomes was due to a modification in the activation of these analogs or to a modification of the nucleotide pools in leukemic cells remained unclear. The present study found a significant correlation between the dCK/cN-II ratio and ara-CTP production in AML cells from patients (Fig. 2). A decrease in this ratio at relapse after ara-C-based chemotherapy was associated with reduced ara-CTP production in the same patient (Fig. 4). In addition, a close association among dCK/cN-II ratio, ara-CTP production, and ara-C sensitivity was obvious in cultured cell lines, given that ara-C-resistant variants showed lower ratios with lower ara-CTP production than the ara-C-sensitive counterpart (Table 3 and Fig. 5). These findings strongly suggest that the alteration of the dCK/cN-II ratio directly influences intracellular ara-CTP production and that a decrease in this ratio might lead to reduced ara-CTP production, resulting in poor clinical outcomes.

Mazzon et al. showed that ara-C was only a very week substrate of purified cN-II [35]. However, the importance of this enzyme with regard to the outcomes of ara-C-treated AML has already been demonstrated in several papers [14,16,18]. Moreover, despite no

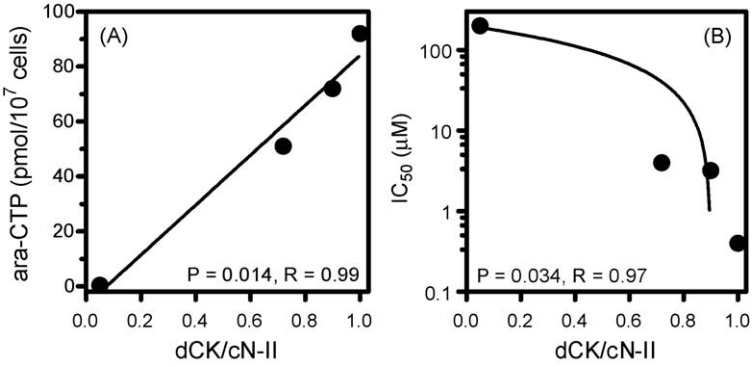

Fig. 5. Relationship between dCK/cN-II transcript ratio and ara-CTP (A) and between dCK/cN-II ratio and ara-C sensitivity (B) in HL-60 cells and its three ara-C-resistant variants (HL-60/R1, HL-60/R2, and HL-60/R3 cells). Intracellular ara-CTP concentrations were determined when cells were incubated with 2 μM ara-C for 6 h. IC_{50} was determined using the XTT assay when cells were incubated for 72 h with different concentrations of ara-C.

direct evidence, our in vitro data using primary leukemic cells and cultured leukemic cell lines suggest participation of cN-II in ara-CTP production. Therefore, Mazzon's report might not completely deny the possibility of cN-II to degrade ara-C phosphate metabolites.

Given the importance of ara-C in induction regimens for AML, the dCK/cN-II ratio may offer a useful predictor for evaluating the sensitivity of leukemic blasts to ara-C. Determination of ara-C sensitivity prior to the initiation of induction treatment and therapeutic drug monitoring of intracellular ara-CTP during the treatment may individualize the chemotherapy for AML patients and improve therapeutic outcomes.

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