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Substrate cycles and drug resistance to 1-beta-D-arabinofuranosylcytosine (araC)

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Abstract
Acute myelogenous leukemia (AML) is the most common form of acute leukemia in adults. After diagnosis, patients with AML are mainly treated with standard induction chemotherapy combining cytarabine (araC) and anthracyclines. The majority of them achieve complete remission (CR) (65 – 80%). However, prospects for long-term survival are poor for the majority of patients. Resistance to chemotherapy therefore remains a major obstacle in the effective treatment of patients with AML. In this review, we highlight the current knowledge of substrate cycles involved in normal deoxynucleoside triphosphate (dNTPs) metabolism and their possible role in drug resistance to araC.

Keywords: *araC, AML, nucleotide metabolism*

Introduction

Acute myelogenous leukemia (AML) is the most common form of acute leukemia in adults with approximately 20,000 new cases and 15,000 deaths caused by this disease each year in the US, Europe and Australia. After diagnosis, patients with AML are mainly treated with standard induction chemotherapy combining cytarabine (araC) and anthracyclines. The majority of them achieve complete remission (CR) (65 – 80%). However, prospects for long-term survival are poor for the majority of patients. In fact, 75 – 80% of patients who achieve their first CR will relapse, and the median time in remission before relapse is only 12 months with current treatments.

Relapse disease is typically treated again with chemotherapy. Unfortunately, subsequent remissions are obtained in only 40 – 60% of relapsed patients who do survive treatment. These remissions have a shorter duration than the prior CR (a median of only 6 months in the case of second patients). Resistance to chemotherapy therefore remains a major obstacle in the effective treatment of patients with AML.

In this review, we highlight the current knowledge of substrate cycles involved in normal deoxynucleoside triphosphate (dNTPs) metabolism and their possible role in drug resistance to araC.

Substrate cycles and nucleotide metabolism

DNA synthesis and repair requires a continuous and balanced intracellular deoxyribonucleoside triphosphates (dNTPs) pool. There are two distinct metabolic pathways which determine nucleotide pools (Figure 1). The first one is named the ''de novo'' pathway and is activated in replicating cells. It is believed that nucleotides thus produced are preferentially used in DNA synthesis. The main step in this pathway is the reduction of ribonucleoside diphosphates into deoxyribonucleoside diphosphates by ribonucleotide reductase (RNR). The second one is the ''salvage'' pathway, which is the main source of dNTPs in resting or G1 cells. This dNTPs will be preferentially used in DNA repair [1,2]. This pathway involves recycling of nucleosides produced by the catabolism of nucleic acids [3]. Extracellular nucleosides are taken up by specific membrane transporters into the cell, and then they are phosphorylated by

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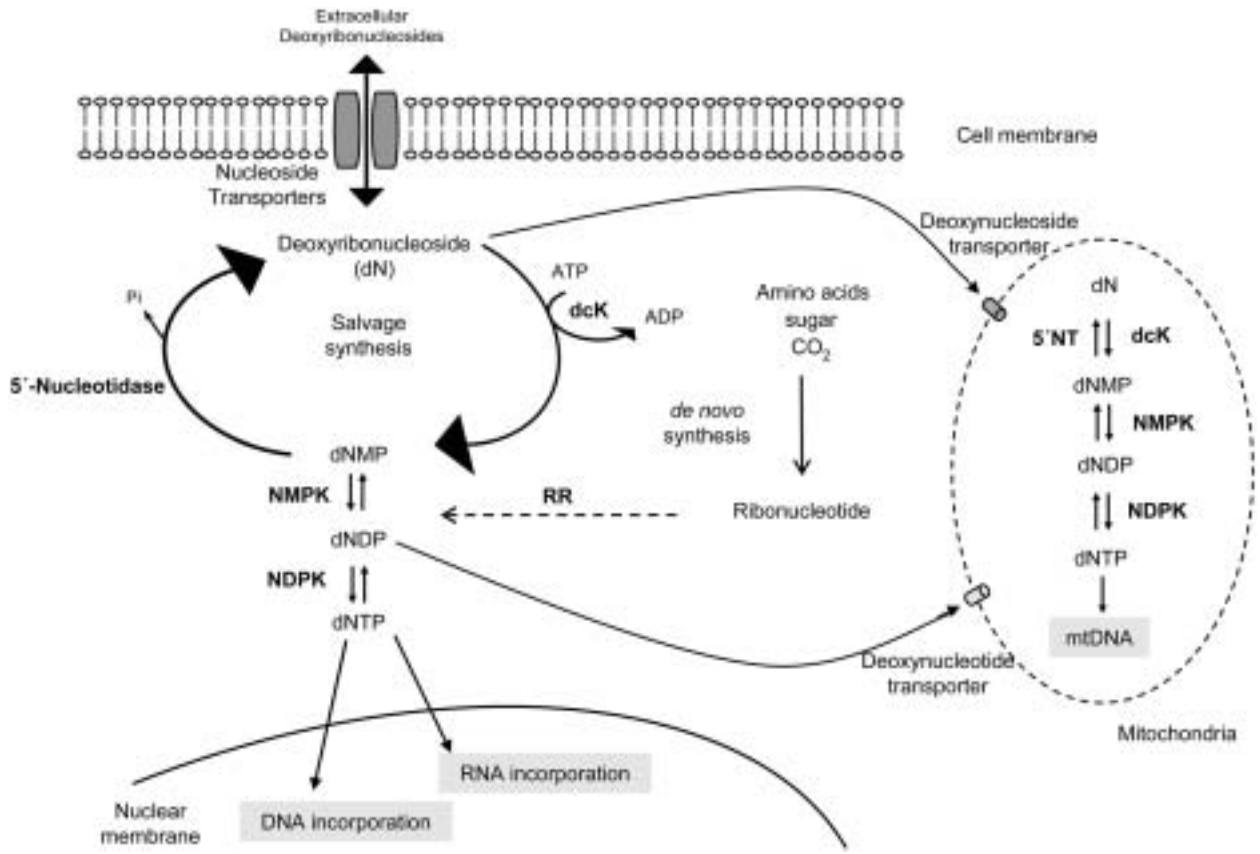

Figure 1. Schematic representation of nucleosides metabolism. Nucleosides are newly synthesized in the de novo pathway or are incorporated into the cell by specific nucleoside transporters through the salvage synthesis. In this case, nucleosides are phosphorylated by dCK, NMPK and NDPK to their phospho-deoxyribonucleosides derivates. Cytoplasmic 5′-nucleotidase activity opposes that of dCK by dephosphorylating monophosphoderivates. Both deoxyribonucleosides and deoxyribonucleosides diphosphate (dNDP) in the cytosol can enter the mitochondria by specific membrane transporters. Finally, deoxyribonucleotides are incorporated into newly synthesized DNA or RNA.

specific nucleoside kinases to become nucleoside monophosphates. In mammals there are six main kinases for this pathway named deoxycytidine kinase (dCK), thymidine kinases 1 and 2 (TK1 and TK2), deoxyguanosine kinase (dGK) and uridine-cytidine kinases 1 and 2 (UCK1 and UCK2). Nucleosides monophosphates are next phosphorylated by monophosphate kinases (UMP-CMPK, thymidylate kinase, guanylate kinase and adenylate kinases) and finally by nucleosides diphosphates (NDPK) kinases to become dNTPs (Table 1).

Given that the intracellular concentration of dNTPs pool must be controlled, they are continuously degraded by one of the two main catabolic pathways. The first one is mediated by intracellular deaminases such as cytidine deaminase (CDA), adenosine deaminase (ADA) and deoxycytidylate deaminase (dCMP-deaminase). These enzymes deaminate non-phosphorylated deoxyribonucleosides and deoxyribonucleoside-monophosphates (MP). The other catabolic pathway is exclusively for monophosphorylated derivatives, as they are substrates for 5′-nucleotidases. The role of the 5′-nucleotidases is the opposite to that of the nucleoside kinases, decreasing the amounts of phosphorylated forms of deoxynucleosides inside the cells. Several human 5′-NTs with different subcellular localization have been cloned and they can be classified in three main classes: membrane-bound, cytosolic and mithochondrial 5′-NTs. The membrane-bound enzyme is termed ecto-5′NT and is also known as CD73, the mitochondrial 5′-NT is called mdN and finally the five 5′-NT in the cytosol are termed cN-I (A and B), cN-II, cN-III and cdN [4].

When dNTPs are needed, deoxyribonucleosides are taken into cells, phosphorylated, and incorporated into DNA. These regulated mechanisms provide the cell with continuous and balanced nucleotide dNTPs pools [3]. If the pool of deoxyribonucleotides exceeds the requirements for DNA replication and repair, the surplus is degraded and leaves the cell as deoxyribonucleosides [5].

Table I. Biochemical characteristics of the main enzymes involved in nucleoside metabolism.

Enzyme	Description	Chromosome localization	Protein size	Principal substrates	Tissue distribution
dCK	Cytosolic deoxycytidine kinase	4q13.3-q21.1	30.5 kDa (260 aa)	Deoxycytidine Deoxyadenosine Deoxyguanosine	Lymphoid and neoplastic tissues
UMP-CMPK	Cytosolic monophosphate kinase	1p31	26 kDa (196 aa)	Uridine-MP Cytidine-MP Deoxyuridine-MP Deoxycytidine-MP	Ubiquitous
NDPK (H1and H2)	Nucleoside diphosphate kinase	17q 21.3	17.15 kDa (H1) (177 aa) 17.3 kDa (H2) (152 aa)	Deoxynucleosides-TP	Ubiquitous Overexpressed in tumors
cN-II	Cytosolic selective 5′-NT	10q24.32	65 kDa (561 aa)	Inosine-MP Deoxyinosine-MP Guanosine-MP Deoxyguanosine-MP Hypoxanthine-MP	Ubiquitous
cN-III	Cytosolic 5′ nucleotidase	7p15.3	36 kDa (286 aa)	Uridine-MP Cytidine-MP	Red blood cells
cdN	Cytosolic 5′(3′) deoxynucleotidase	17q25.3	23.9 kDa (200 aa)	Deoxyguanosine-MP Deoxyinosine-MP	Ubiquitous
CDA	Cytidine deaminase	1p35-p36.2	16.1 kDa (146 aa)	Cytidine Deoxycytidine	High in lymphoid cells ND

Substrate cycles and araC metabolism

The metabolism and the mechanism of action of araC are based on the metabolism of physiological deoxyribonucleosides. In fact, araC enters cells by using the equilibrative nucleoside transporter 1 (hENT1) and undergoes intracellular phosphorylation by deoxycytidine kinase (dCK) (Figure 2). This first phosphorylation step is the limiting step in intracellular araC activation, and dCK activity is therefore crucial to obtain any cytotoxic effect. The monophosphorylated metabolite of araC, araCMP, is further di- and tri-phosphorylated by kinases of the salvage pathway, to obtain araCTP [6]. This active form of araC acts as an antimetabolite, competing with physiologic nucleosides for the incorporation into DNA which results in synthesis inhibition and chain termination. In addition, araCTP interacts with DNA polα. These different effects may lead to cell death by apoptosis. araC metabolites might also be substrates for catabolizing enzymes such as deaminases and 5′-NTs [7,8]. An increase of the activity of such enzymes might decrease the accumulation of araCTP and therefore the cytotoxic effect of araC (see below).

araC activating kinases

Deoxycytidine kinase is the main cytosolic enzyme that catalyzes the initial step of the 5′-phosphorylation of three of the four natural deoxyribonucleosides: deoxycytidine (dC), deoxyguanosine (dG) and deoxyadenosine (dA). dCK is also an essential enzyme for the phosphorylation of araC as well as other nucleoside analogues (NA) to their monophosphate form [9,10], thus playing an important role in the activation of NA. dCK can use different NTPs as phosphate donor in the phosphorylation of deoxycytidine, but seems to prefer UTP over other NTPs [11–14]. Overall dCK activity is known to be under a feedback control by dCMP, dCTP and ADP [15].

Human dCK gene is localized in chromosome 4. It is codified by 7 exons under the control of ubiquitously expressed transcription factors such as Sp1 and USF1/USF2 [16]. These interactions could regulate dCK expression over a wide range and contribute to tissue-specific patterns of expression of this gene. dCK functions as a 60 kDa homodimer, consisting of two identical subunits of 30,5 kDa each [17]. It has been demonstrated that dCK is a constitutive enzyme and its activity is highly variable (between 2–10

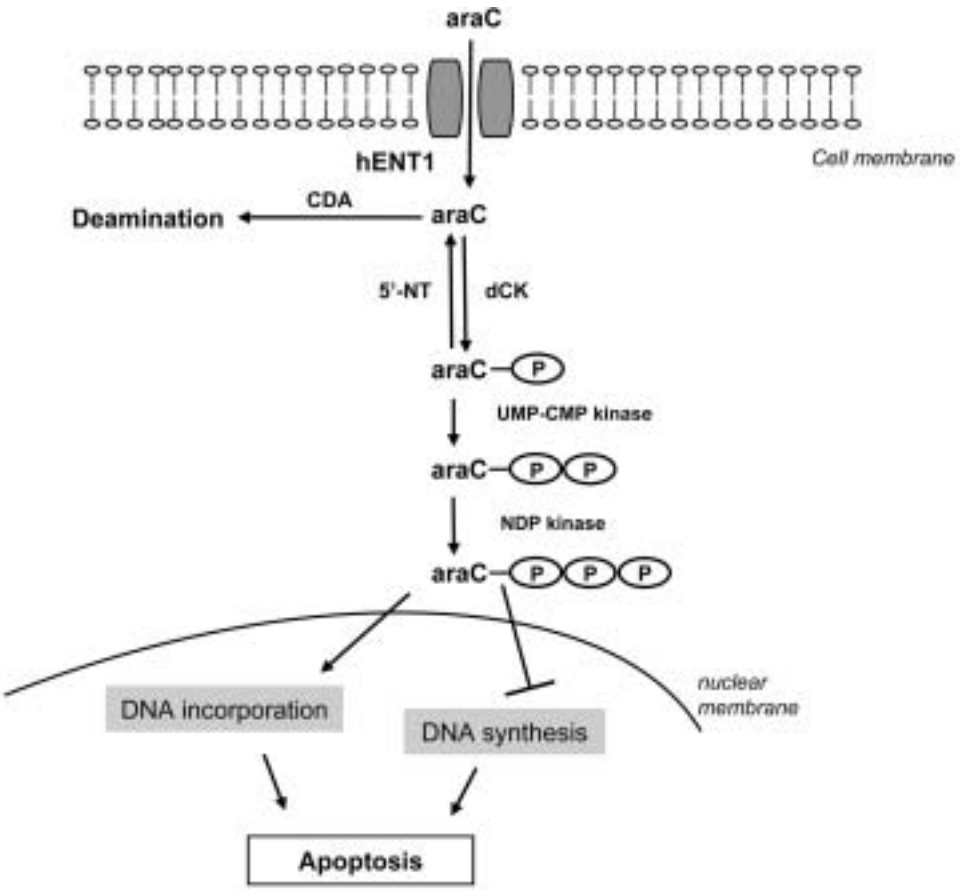

Figure 2. Metabolism and mechanism of action of the nucleoside analog araC. araC enters the cell through specific nucleoside transporters and is activated by phosphorylation to its triphosphate derivate. araC catabolism results from rapid deamination to non-toxic metabolites. Active araC exerts its action by being incorporated into and altering the DNA or by interfering with enzymes involved in DNA synthesis leading to cell apoptosis.

times) in extracts of different tissues. dCK activity is high in lymphoid, mononuclear blood cells and quiescent cells in which it phosphorylates nucleosides needed for DNA repair, increasing its activity several-fold when cells enter the S-phase of the cell cycle [18–21]. The correlation between the expression of dCK mRNA, protein and the enzymatic activity using a panel of solid tumors, leukemic and lymphoma cell lines has been addressed by van der Wilt et al. [22] and more recently by Sigmond et al. [23]. It was found that the expression of dCK mRNA was closely correlated to the activity of this enzyme.

The nucleoside monophosphate kinase UMP-CMP kinase (UMP-CMPK) is involved in the phosphorylation of UMP, CMP, dUMP and dCMP to their respective nucleoside diphosphates. UMP-CMPK seems to be also the nucleoside monophosphate kinase implicated in araCMP phosphorylation [6,24]. This enzyme is a 26 kDa cytoplasmic nuclear kinase that was cloned in 1999 [24]. ATP and dATP are the main phosphate donors for this enzymatic reaction.

The ultimate phosphorylation of nucleosides is catalyzed by nucleoside diphosphate kinases (NDPKs or nm23). This is a group of eight proteins in which five have shown nucleoside kinase activity with ATP or GTP as phosphate donor (see [25] for recent review). Isoforms H1 and H2 seem to play an important role in the production of araCTP [26]. However, the implication of these enzymes in the production of dNTPs is discussed because of the high affinity towards NDPs as compared to dNDPs. araCDP is phosphorylated by purified NDPK from human cells [26], and therefore this enzyme is likely to be involved in intracellular araC metabolism.

5′-nucleotidases implicated in araC metabolism

As for natural nucleosides, 5′-NTs might oppose the phosphorylation of NAs. Three of the known 5′-NTs have been shown to modulate araC activity in cell models or in patients treated for AML. cN-II was the first human cytoplasmic 5′-NT cloned from placenta

[27]. Human cN-II gene is located in chromosome 10q24.32, and for vertebrate tissues, active cN-II is a homo-tetramer in which each subunit has a molecular mass of 60–70 kDa [28–30]. This enzyme is an IMP/GMP-selective 5′-NT and its activity opposes the action of the salvage enzymes by dephosphorylating IMP and GMP, thus participating in the regulation of purine deoxyribonucleotide metabolism (ATP and GTP).

cN-II expression is ubiquitous in mammals and it is present in different organs and tissues [31]. High enzyme activity has been measured in tissues with a rapid turnover rate of nucleic acids or DNA synthesis such as testis, liver, spleen and lymphoblastoid cells. By contrast, very low levels of cN-II activity were found in skeletal muscle and erythrocytes [32,33].

The enzymatic activity of cN-II requires the presence of Mg^{2+} and a pH optimal between 6 and 7.5 [34]. cN-II activity is stimulated by ATP [30] and regulated/inhibited by its substrates inosine/guanosine and by inorganic phosphate possibly by subunit association-dissociation [30]. It has been proposed the formation of an enzyme-phosphate intermediate after the hydrolysis of the phosphate group in nucleoside monophosphate. This intermediate could transfer the phosphate moiety to water or to a nucleoside [35]. In this latter case, cN-II acts as a phosphostransferase, and it has been shown that by the means of this activity, it is able to phosphorylate antiviral nucleoside analogues [35–38]. However, there is no evidence showing that cN-II actually phosphorylates NA currently used in anticancer therapy. Thus, it seems that cN-II acts as nucleotidase or phosphotransferase, playing catabolic or anabolic functions respectively depending on the concentration of inorganic phosphate, ATP and the availability of a suitable nucleoside, which is a better phosphate acceptor than water [36].

Another 5′-nucleotidase, cN-III (also called pN-I), was cloned and characterized in 2000 [39]. The gene situated on chromosome 7p15.3 encodes a 36 kDa protein which dephosphorylates preferentially UMP and CMP as well as the monophosphorylated form of araC [39]. Mutations in cN-III might cause protein deficit and hemolytic anemia [40–43].

Finally, cytoplasmic deoxynucleotidase (cdN also called dNT-1 or pN-II) was cloned in 2000 by Rampazzo et al. [44]. Its gene is situated on chromosome 17q25.3 and encodes a 23.9 kDa protein containing 5′-nucleotidase activity when forming a homodimer. cDN prefers deoxyribonucleotide monophosphates and monophosphates of 5′-deoxyribonucleotides over 3′-deoxyribonucleotides as substrates in its dephosphorylating enzymatic reaction. Purified cdN is not capable to dephosphorylate araCMP, but this enzyme could be involved in araC metabolism by the means of substrate cycles in AML cells [45].

Deaminases involved in araC metabolism

As cited before, deaminases are involved in dNTP metabolism by deaminating nucleosides or monophosphorylated forms. Cytidine deaminase (CDA) is a 48.7 kDa protein encoded by a gene situated on chromosome 1p35-p36.2 [46–48]. It transforms dC and C into dU and U respectively, as well as araC into araU [49]. Different CDA inhibitors have been identified, such as THU (3,4,5,6-tetrahydrouridine), zebularine, 5-fluorozebularine and diazepinone [9,49].

dCMP deaminase is another protein involved in nucleotide metabolism by deamination. Its gene located on chromosome 4q35 and, cloned in 1993, encodes a 20 kDa protein capable of transforming dCMP into dUMP [50]. Its activity is stimulated by dCTP and inhibited by dTTP and THU. No deamination of araCMP by dCMP deaminase has been identified, but it is obvious that this enzyme is implicated in nucleoside metabolism. In fact, dCMP deaminase deficient cells have great perturbations in dCTP pools and dTTP pools [51–54]. These modifications induce an increased rate of spontaneous mutations [55].

Substrate cycles and *in vitro* araC resistant models

Drug resistance to araC is one of the major problems in the treatment of acute leukemias, and therefore it has been largely studied and several mechanisms have been identified. These involve alterations in the structure, expression level or activity of different proteins that play important roles in the metabolism (transporters, kinases, nucleotidases, deaminases) and/or in the cytotoxicity exerted by NA. Alteration on any of these proteins could theoretically induce resistance. In general, araC resistant leukemia cell lines are cross resistant to other NA [56]. In addition to decreased or increased activity of kinases or catabolic enzymes involved in araC metabolism, other mechanisms for resistance could be altered such as intracellular pools of deoxynucleotides, decreased nucleoside transport into the cells, as well as modifications in the cellular apoptotic machinery (for recent review see [57]).

Kinase deficiency

Many studies have reported the development and the characterization of *in vitro* leukemia models for araC

resistance. A large majority of these models shows modification in dCK as evidenced by decreased genomic expression [58,59], kinase activity [60–66] or araCTP accumulation [67,68]. The role of dCK deficiency in this kind of models has been validated by transfection studies. In fact, increased expression of dCK gene by transfection into dCK deficient leukemia cells restored the sensitivity to araC [69]. In addition, specific dCK inhibition by the addition of dCK antisense oligonucleotides in K562 cells turns tumor cells resistant to araC cytotoxicity underlining the direct role of dCK in araC resistance [70]. Beauséjour et al. showed that it is necessary to diminish dCK activity more than 18% to obtain araC resistance, suggesting an excess of dCK in cells treated with NA [71].

Structural analyses of the dCK gene have shown inactivating mutations and deletions that causes dCK deficiency [65] (Figure 3). In leukemic blasts from relapsed/refractory AML patients, Flasshove et al. have shown that among 16 patients who were resistant to araC treatment, 7 present point mutations in dCK gene. However, only one of them had a mutation that inactivated the enzyme [72] raising the question of the clinical relevance of these findings. More recently, van den Heuvel-Eibrink et al. show that dCK point mutations are not frequently observed and that they have no clinical relevance in the araC resistance in a study performed in 30 AML patients [73]. DNA methylation has also been suggested as a possible mechanism of dCK gene regulation. On the other hand down-regulation of dCK gene may also come from inhibition of gene transcription by steroids. Exposure of human cancer cells to cortisol or dexamethasone partially reduced gemcitabine cytotoxicity [74,75]. As steroids are used in cancer treatment, it is probably that they can modify NA cytotoxicity by decreasing dCK transcription.

Another study made in multidrug resistant K562 cell line developed for resistance to daunorubicin, which is used in association with araC in treatment of leukemia, showed 15-fold cross-resistance to araC associated with a three-fold decrease in dCK activity [76]. There was no difference in dCK mRNA expression level, thus suggesting a gene alteration or post-transcriptional or translational events to cause the decrease in dCK activity.

Post-translational modifications of enzymes activities are numerous. One possible explanation is the activation of dCK by protein phosphorylation. This possibility is supported by results from Wang and Kucera [77] who found that the activation of dCK was increased by *in vitro* protein kinase C treatment. Moreover, results from Csapó et al. [78] showed that treatment of cells with NaF, an inhibitor of protein phosphatases, increases dCK activity while inhibits DNA synthesis. By contrast, the addition of phos-

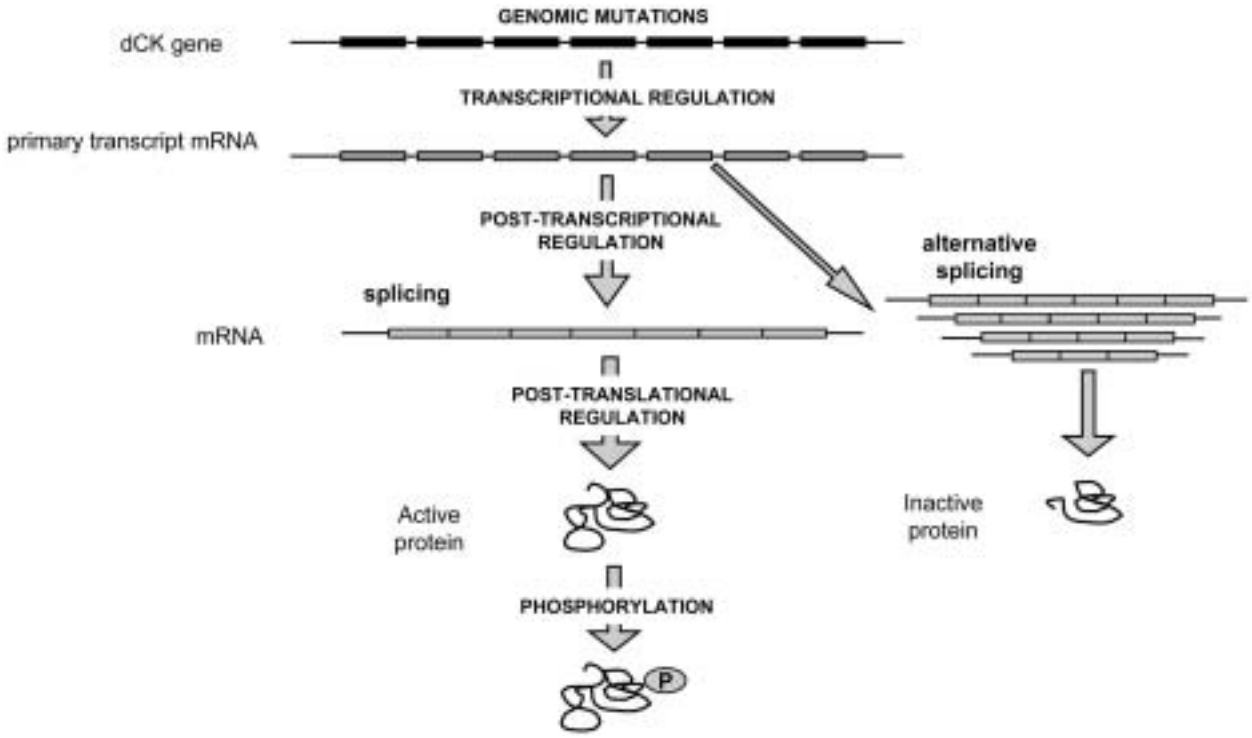

Figure 3. Cellular processes responsible for dCK regulation associated to araC resistance.

phatases inhibited dCK activation, showing that this effect is a result of dCK phosphorylation [79].

A number of cytotoxic drugs, such as etoposide, cladribrine, araC, fludarabine and clofarabine have been found to stimulate dCK activity in human and murine leukemic cells [80,81]. It is important to notice that this stimulation requires a basal dCK level in the cells, because in NA-resistant and dCK-deficient cells there was no stimulation of the enzyme (unpublished data from Galmarini).

Finally, four different alternatively spliced dCK forms of mRNA were identified in coexpression with wild-type (wt) dCK in purified leukemic blasts from patients with clinically resistant AML [82] without any modification on the dCK gene. *In vitro* activity assay showed that these variants of dCK were inactive, with lower molecular weights than wt dCK. More importantly, these proteins were unable to restore araC sensitivity in dCK-deficient rat leukemia cells when transfected alone or with wt dCK [83]. However, when transfection was done into araC sensitive cells, these spliced dCK forms did not change sensitivity towards araC [76] indicating that they cannot act as dominant-negative inhibitor on dCK wild type cells when they are coexpressed in a single cell [83]. In any case, alternative spliced dCK forms might contribute to araC resistance in patients with AML if wt dCK expression is lost, as was demonstrated *in vitro* [82].

In reference to the other kinases, no *in vitro* model for araC resistance has shown alterations in the activity or expression levels of UMP-CMPK and NDPK.

Increased expression/activity of deaminases and 5′-nucleotidases

It is interesting to notice that no *in vitro* model for araC resistance developed by continuous exposure to the drug with increased deaminases has been reported. However, the implication of CDA in araC activity has been evidenced by the resistance induced by CDA-transfection in leukemic CCRF-CEM cells [84]. In fact, several CDA transfection studies show a decreased sensitivity to araC in overexpressing fibroblasts, and Neff et al. proved this in a leukemic cell line [84]. dCMP deaminase could theoretically also be involved in this mechanism, but no published data is available on this subject.

In reference to 5′-nucleotidases, *in vitro* models using cancer cell lines have demonstrated that intracellular level of cN-II seems to influence cell sensitivity to NA [56,85,86]. In the CCRF-CEM cell line, it has been shown that cross-resistance with cladribrine, araC and gemcitabine was associated to a 2-fold higher level of cN-II, higher dCTP levels and normal dCK level [86]. Schirmer et al. also reported that cladribrine resistant HL60 cells had a 2–3-fold increased activity of cN-II comparing with its parental sensitive cell line, without any change in dCK activity [85]. However, the overexpression of cN-II in 293 human embryonal kidney cells did not induce resistance to cladribrine [87], neither has any araC resistant cell model shown an overexpression of cN-II as mechanism for the resistance. There are no published data concerning gene mutations or post-translational modifications that might increase cN-II activity and be responsible for the phenotypic changes observed in resistant cell lines [57]. To date, any study has demonstrated the origin of overexpression of cN-II activity and mRNA in resistant cell lines. The role of cN-II in NA resistance remains to be elucidated, because there is no data showing that cN-II can dephosphorylate the different NA [45].

Another 5′-NT, cN-III, is able to dephosphorylate araCMP [8]. However, whether its overexpression can induce araC resistance has not yet been proved. As for cN-II, no *in vitro* data on the implication of cdN in araC resistance exist.

Substrate cycles and araC resistance in the clinic

Given that activating and catabolic enzymes involved in substrate cycles interact with a large variety of NA used in anticancer therapy, their activities may have high relevance to the therapeutic effectiveness in different types of leukemia [88].

Development of resistance during chemotherapy can often be the result of inactivation of dCK and/or increased activity of cN-II. It was early reported that dCK activity correlated with the response to araC treatment in 21 patients with AML [89]. Similarly, ALL patients more often relapsed when dCK expression was low or absent [90]. However, activation of NA by dCK is not always indicative of *in vivo* response to these NA [91], (for review see [92]). Thus, the role of this enzyme in clinical drug resistance remains a subject of controverse. In reference to UMP-CMPK or NDPK, up to date, there is no clinical information regarding their relevance in the therapeutic effectiveness in different types of leukemia.

Concerning cN-II, some studies have demonstrated that there is a correlation between cN-II activity and resistance to NA [93]. High levels of cN-II activity were correlated to a worse clinical response in acute and chronic leukemias: In ALL, high levels of cN-II activity was associated with *in vitro* thiopurine resistance [94], while in AML there is a correlation between high cN-II mRNA levels and araC resistance and worse clinical outcome [95–97]. Moreover, the pretreatment levels of cN-II were

significantly lower in responders than in non-responding patients. This study also shows that patients whose blasts had detectable expression of cN-II at diagnosis and relapse had a shorter disease-free survival (DFS) and overall survival (OS) than cN-II-negative patients [95], suggesting a role for cN-II in the *in vivo* activity of araC in AML patients. Rather than an isolated increase in cN-II or a decrease in dCK, an increase in the ratio of cN-II to dCK (cN-II/dCK) seems to be a good indicator for resistance to NA. We have recently reported that in AML patients a high cN-II/dCK ratio was related to a worse clinical outcome [98]. The relative ratio may have a predictive clinical value with cN-II contributing to drug resistance.

Clinical evidences for a role of cdN in araC resistance have been published recently [99]. Cells overexpressing this 5′-NT produce more dC, dU, U, C and T than control cells, showing that this enzyme is involved in cell cycles regulating nucleosides [100]. Finally, the implication of cN-III *in vivo* araC resistance is not known.

The *in vivo* correlation between CDA activity and araC resistance remains controversial and the relative contribution of CDA to drug resistance has not yet been fully elucidated. The clinical relevance of a high level of CDA activity as a major cause of araC resistance in AML patients has been emphasized by several investigators [89,101 – 103]. Conversely, we and others did not find a relationship between increased CDA mRNA expression or enzyme activity and resistance to therapy in AML patients [96,104]. Structural analyses of the CDA gene showed the correlation between a polymorphism at codon 27 and substantially different deamination rates of araC *in vitro* [103]. This structural aberration did not seem to represent a major cause for the differences observed in CDA activities between araC sensitive and araC resistant patients. Schröder and co-workers demonstrated a significant correlation between the amount of CDA mRNA and CDA enzyme activities in AML blasts suggesting that variations in CDA activity result from differences in gene expression [105]. It therefore appears that CDA activity *in vivo* is correlated with transcriptional regulation rather than with CDA gene aberrations.

Conclusions

AML therapy usually fails because of the appearance of drug resistance [106]. Actually, response following araC induction regimens is variable and is dictated by a set of well-described prognostic factors. Performance status and age are the principal predictors of early death, whereas cytogenetics and history of abnormal blood counts are predictors of resistance. In fact, rates of therapy-induced mortality increase with increasing age, abnormal organ function, and particularly, poor performance status. However, standard therapy is satisfactory only for a minority of patients.

Thus, there is a need for new prognostic and predictive factors that could be used for planning of more effective treatments. A realistic goal would be the identification of tumor cell traits which could allow tailored or targeted therapy. In this sense, a better knowledge of metabolic pathways involved in araC activation would help to determine the expression of a phenotype of probable resistance to araC, that would be of great value in the choice of optimal therapy, and the avoidance of administration of ineffective but toxic treatments.

A better knowledge of the role of substrate cycles in araC activation would also help to optimize the efficacy of araC-based combinations and to develop more potent araC derived compounds that are less sensitive to the resistance mechanisms described above. Efforts to increase intracellular levels and DNA incorporation of phosphorylated araC are very promising [107,108]. In the same way that combination chemotherapy has provided curative treatment of AML, a multifactorial approach of araC resistance should allow significant progress in the treatment of currently chemoresistant disease.

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