2 The Role of Deoxycytidine Kinase in DNA Synthesis and Nucleoside Analog Activation

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SUMMARY

Deoxycytidine kinase (dCK) is the main enzyme in the salvage of deoxyribonucleosides as a consequence of its broad substrate specificity. dCK is the only enzyme that can supply cells with all four precursors of DNA; is capable of 5′-phosphorylation of the natural substrates deoxycytidine (dCyt), deoxyadenosine, and deoxyguanosine; and can be interconverted into

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thymine nucleotides. The deoxycytidine triphosphate (dCTP), in addition to DNA, can be utilized for special processes, such as for synthesis of "liponucleotides," which are precursors of membrane phospholipids. The expression of dCK is highest in lymphoid cells/tissues (e.g., such as thymus, spleen, lymph nodes, stimulated peripheral blood mononuclear and bone marrow cells) and in all malignancies of these cells. The cell cycle dependence of the expression of dCK has been a matter of discussion; even higher dCK activity and dCyt metabolism were found in undifferentiated rather than in differentiated human lymphocytes. An enhancement of dCK activity occurred on preincubation of cells with a variety of nucleoside derivatives and nonnucleoside genotoxic agents, such as aphidicolin, etoposide (VP16), taxol, and even the G protein modulator sodium fluoride. γ-Irradiation and ultraviolet (UV) C irradiation also augmented dCK activity in different cells. The decrease of dCK activity was observed with protein phosphatase inhibitors, suggesting a regulatory role for reversible protein phosphorylation in the activation process. Cytosolic Ca^{2+} ion and p53 protein are necessary for the increase of dCK activity in cells after toxic treatments. The reason for the increase of dCK activity after toxic treatment of cells seems to be a compensatory mechanism induced by "metabolic stress" signals; cells need deoxynucleotides to repair damaged DNA. A positive correlation was found between dCK activity and the sensitivity of malignant cells to chemotherapy; thus, dCK has an outstanding importance in human chemotherapy. dCK is often the rate-limiting enzyme in the activation of these analogs. L-2′3′-dideoxy-3′-thiacytidine (lamivudine, 3TC); arabinosylcytosine (Cytosar, ara-C); 2-chlorodeoxyadenosine (cladribine, CdA); and 2′,2′-difluorodeoxycytidine (gemcitabine, dFdC), the first a human immunodeficiency virus drug and the last three valuable anticancer agents, are all substrates for dCK, and they are between 5% and 50% as efficient as dCyt as substrates for the enzyme. dCK prefers nucleoside sugars in the S-conformation (C2′-endo-C3′-exo) because α-2′,3′-dideoxycytidine adopts that conformation preferentially. dCK is composed by two identical polypeptides of 261 amino acids *(54)*, and it shows some significant sequence similarity with the herpes simplex type 1 virus thymidine kinase, as well as about 40% sequence identity to the mitochondrial thymidine kinase 2. In 2003, the structure of dCK in complex with dCyt and ADP-Mg^{2+} was solved.

The activated form of dCK seems to be an altered stable conformation of the enzyme in which the C-terminal is differently exposed to immunoreagents. Further studies are needed to define the molecular mechanism responsible for the activation process, but it is clear that increased understanding in this field may lead to the development of new drug combinations in antitumor or antiviral chemotherapy. With determination of the structure of dCK, it is now possible to define some of the structure function relationships of this enzyme and the related expanding deoxynucleoside kinase enzyme family.

Key Words: Deoxycytidine kinase; deoxyguanosine kinase; deoxynucleoside analogs; deoxynucleoside kinases; deoxynucleosides; thymidine kinases.

1. INTRODUCTION

This chapter mainly concerns the role of deoxycytidine kinase (dCK; EC 2.7.1.74) in cellular nucleotide metabolism and in activation of nucleoside analogs used in chemotherapy. However, Chapter 3 also describes the activation of many of the most important deoxynucleoside analogs by the four cellular deoxynucleoside kinases (dNKs). We therefore focus on the special metabolic function of dCK in relation to metabolism and DNA synthesis; refer to Chapter 3 and to several earlier review articles *(1–4)* for a more comprehensive account of the topic. Here, we summarize the basic facts regarding dCK but focus on the results obtained during recent years.

The adequate maintenance of intracellular deoxyribonucleotide pools to supply the needs of deoxyribonucleic acid (DNA) replication, repair, and recombination is a central issue of nucleotide metabolism, accomplished by the tightly regulated *de novo* and salvage biosynthetic pathways presented in Fig. 1. Purine and pyrimidine ribonucleotides can be synthesized from carbohydrate and amino acid derivatives by the energy consuming *de novo* biosynthetic pathways producing ribonucleoside diphosphates (NDPs), which are reduced by the ribonucleotide reductase (RR) to the corresponding deoxyribonucleotide diphosphates (dNDPs) and converted to deoxyribonucleotidetriphosphates (dNTPs).

The synthesis of deoxythymidine 5′-triphosphate (dTTP) is slightly different from that of the others; deoxyuridine monophosphate (dUMP) is methylated by thymidylate synthase to deoxythymidine monophosphate (dTMP) and then phosphorylated to deoxythymidine triphosphate (dTTP) by kinases. However, the mitochondria and some tissues (i.e., erythrocytes, polymorphonuclear leukocytes, peripheral lymphocytes, and brain) in mammals have reduced capacity for *de novo* synthesis of nucleotides; therefore, they are dependent on exogenous bases or nucleosides (salvage processes) to supply the ribo- and deoxyribonucleotides required for different metabolic processes and nucleic acid synthesis. The main sources of deoxyribonucleotides in the cytosol and in the mitochondrion are presented in Fig. 1.

Nucleosides are transported through the plasma and mitochondrial membranes by different nucleoside transporters and immediately phosphorylated by the cytosolic or mitochondrial dNKs. Two cytosolic dNKs (dCK and TK1) and two mitochondrial dNKs (deoxyguanosine kinase [dGK] and thymidine kinase 2 [TK2]) have been identified in different cells and tissues (Fig. 1).

Among different salvage enzymes in different tissues, dCK is the main enzyme in the salvage of deoxyribonucleosides as a consequence of its broad substrate specificity. The dCK has an outstanding importance in

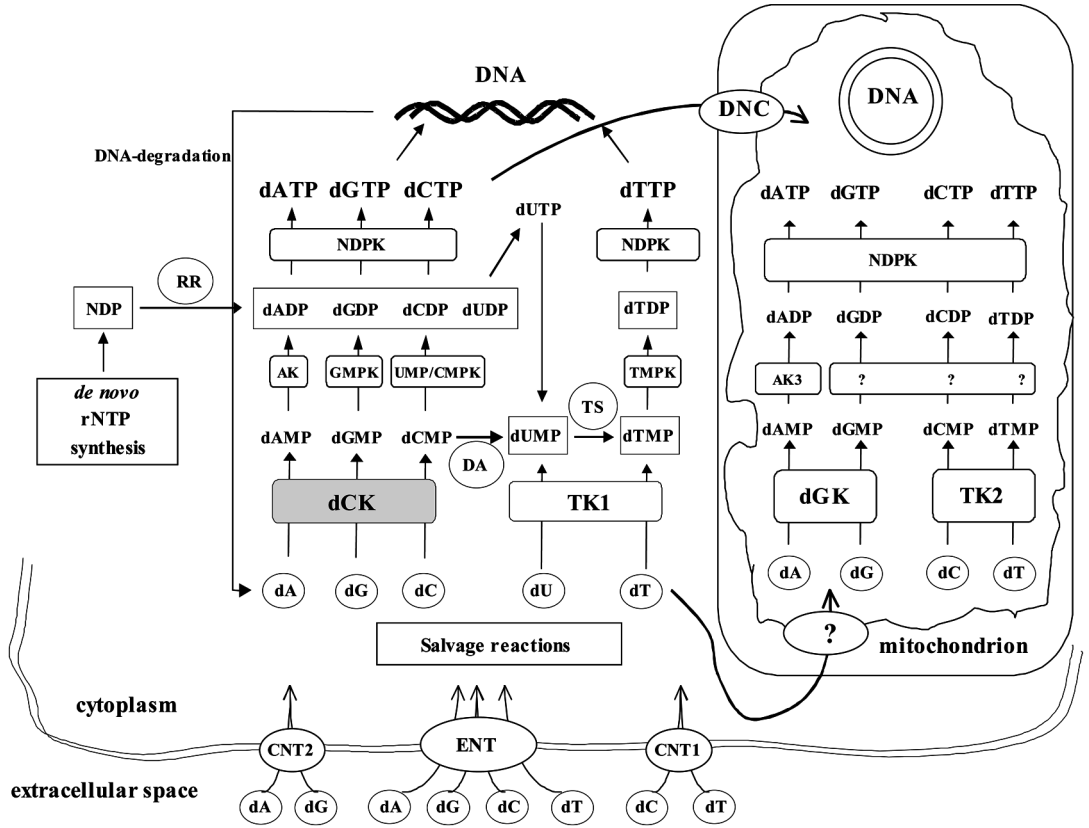

Fig. 1. The sources of deoxyribonucleotides in the cytoplasm and mitochondrion. CNT, concentrative nucleoside transporter (NT); ENT, equilibrative NT; DNC, mitochondrial deoxyribonucleotide carrier; RR, ribonucleotide reductase; DA, dCMP deaminase; TS, thymidylate synthase; AK, adenylate kinase; GMPK, guanylate kinase; UMP/CMPK, TMPK, UMP/CMP kinase and TMP kinase; NDPK, nucleotide diphosphate kinase.

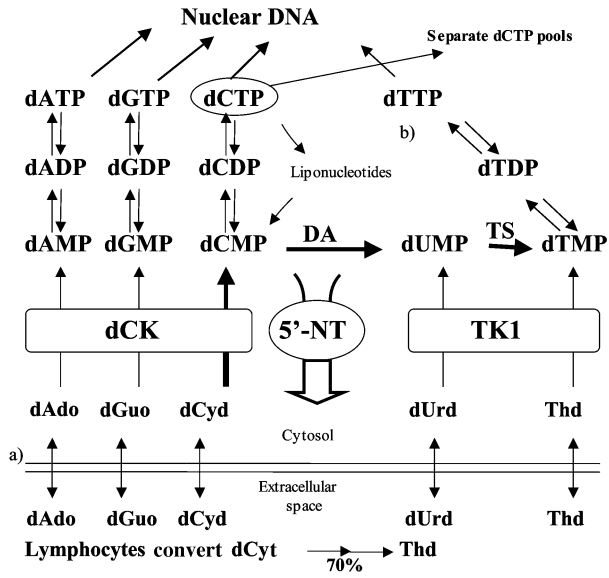

Fig. 2. Salvage of deoxynucleosides into liponucleotides, the separate dCTP pools. dCyt, deoxycytidine; 5′-NT, 5′-nucleotidase.

human therapy because it is also responsible for the activation of many important nucleoside derivatives used in anticancer and antiviral therapy. The enzyme is capable of 5′-phosphorylation of the natural substrates deoxycytidine (dCyt), deoxyadenosine (dAdo), and deoxyguanosine (Fig. 2) and a large number of both pyrimidine and purine analogs *(1–3)*. The physiological phosphate donor for the reaction is most likely both adenosine triphosphate (ATP) and uridine triphosphate (UTP), and the nature of the phosphate donor affects the kinetics of the reaction (*1–4* and references therein). There exists an interconversion pathway between dCyt and deoxythymidine (dThd) nucleotides in the cytosol. The main enzyme in the dCyt-dThd interconversion pathway is the deaminase activity (DA); in the catabolic pathway, it is the nucleotidase activity (5′-NT) presented in Fig. 1.

The metabolic importance of dCK is apparent from the observations that, in some mammalian cells, the major source of dTTP comes also from its product dCMP via dCMP deaminase (dCMP-DA) rather than from deoxyuridine monophosphate via ribonucleotide reduction *(4–6)* as presented in Fig. 2. Approximately 75% of extracellularly added dCyt was converted into dTTP via dCMP-DA and TS in human lymphocytes *(7)*. The importance of the other products, dAMP and dGMP, is less clear because in most situations there is efficient catabolism of purine deoxynucleosides. Only with stable purine analogs or inherited deficiencies of catabolic enzymes is there a significant accumulation of these type of dCK

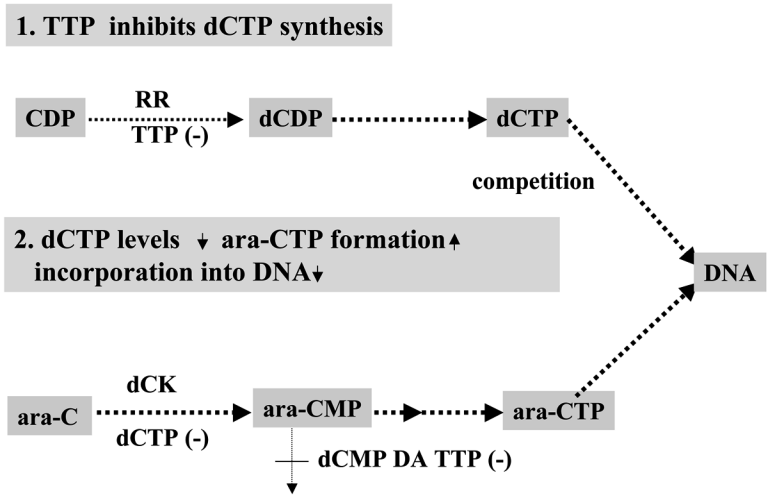

Fig. 3. The regulation of dCTP synthesis and ara-C phosphorylation.

products. The synthesis of dCMP directly by dCK became a central role in supply of all processes utilizing only dCyt for special functions (i.e., for phospholipids) and the other three deoxynucleotides for DNA as seen in Fig. 2.

dCK is the only enzyme that can supply cells with all four precursors of DNA. The final product of the salvage, beside of DNA *(8–10)*, the deoxycytidine triphosphate (dCTP) can also be utilized for special processes, for synthesis of "liponucleotides," precursors of membrane phospholipids *(11–14)*. Treatments of patients with dCyt analogs often resulted in neuropathy as a side effect, which might be the result of altered phospholipid biosynthesis. dCyt is incorporated into dCDP-ethanolamine and dCDP-choline more effectively than cytidine itself, and the anticancer analog arabinosyl-cytosine also participates in these reactions *(11–13)*. The inositol-phospholipid pathway, deoxycytidine diphosphate (dCDP)-diacylglycerol can also incorporate external dCyt, but only in the presence of the amphipathic, neuroleptic drug chlorpromazine *(12,14)*, inhibiting its further metabolism. The incorporation of dCyt into two separate dCTP pools and into membrane phospholipids is presented in Fig. 2.

An important function of dNTP pools has been demonstrated in the activation of origenes during DNA replication *(10)*, and dCK is involved in the overall regulation of the dNTP pools in most cells. The concentrations of dCTP and dTTP pools are regulated at different levels of the metabolism, which then influences the activation processes and effects of nucleoside analogs used in cancer chemotherapy. The inhibition of the dCTP synthesis by the allosteric effect of dTTP occurs at the level of RR, where the reduction of CDP is regulated by dTTP (Fig. 1). There is

	dCK	TK1	TK2	dGK
Resting PBMC	**15**	**<1**	**20 - 40**	**20 - 50**
Stimulated PBMC	**30**	**100**	**≈ 100**	**20 - 50**
CEM	**30**	**100**	**≈ 100**	**20 - 50**
Thymus	**100**	**25**	**≈ 100**	**20 - 50**
Brain	**<1**	**<1**	**≈ 100**	**≈ 100**
Muscle	**<1**	**<1**	**10 - 20**	**20 - 50**
Liver	**<1**	**<1**	**20 - 50**	
Colon	**5 - 10**	**5**	**50**	

Fig. 4. Comparison of dCK activities in different tissues.

another regulation step in the formation of dCTP at the level of dCK; that is, at a high level of dCTP there is no further phosphorylation of dCyt by dCK. However, this regulation will also influence the activation of the antileukemic nucleoside analog(s) arabinosyl-cytosine (ara-C). A high level of dCTP will inhibit the phosphorylation of ara-C, decreasing the effect of the drug on DNA synthesis as presented in Fig. 3.

2. THE EXPRESSION OF dCK IN DIFFERENT CELLS AND TISSUES

The expression of dCK is highest in different lymphoid cells/tissues, such as thymus, spleen, lymph nodes, stimulated blood mononuclear cells, and bone marrow cells and in all malignancies of these cells as presented in Fig. 4 *(1,2,7)*. The dCK levels were intermediate in proliferating epithelial cells (lung, colon, placenta) and in resting peripheral blood mononuclear cells and very low in terminally differentiated tissues such as brain, liver, kidney, and muscle (Fig. 4). The TK1 isoenzyme is a cell cycle-regulated enzyme; its activity is high in all proliferating cell types (Fig. 4). The mitochondrial TK2 and dGK isoenzyme activities are mainly constant in different tissues (Fig. 4).

There is a discrepancy between the relatively high messenger ribonucleic acid (mRNA) levels and the lack of enzyme activity in adult brain extracts *(3)*. However, embryonic sympathetic neuron cultures are inhibited by nucleoside analogs, such as ara-C *(15)* and cladribine (2-chlorodeoxyadenosine, CdA), a dAdo derivative *(16)*, which is an excellent substrate for dCK *(3)* in vitro. The effect of CdA on neurons could be reversed by dCyt, indicating

that phosphorylation by dCK is a necessary step in the nucleoside toxicity observed in embryonic neuron cultures *(16)*. The conclusions from these results might be that, during cell differentiation, there are also fundamental changes in the capacity of the nucleoside metabolic pathways.

The cell cycle dependence of the expression of dCK was a matter of discussion. There was only a slight (two- to threefold) increase in dCK activity in S phases (i.e., large lymphoid cells compared to small, e.g., G1 phase cells) *(1–4)*. Surprisingly, even larger differences were found in dCK activity and dCyt metabolism between undifferentiated and differentiated human tonsillar lymphocytes *(17)* and between cell fractions isolated from germinal centers of lymph nodes, where B lymphocyte differentiation occurs *(18)*. These differences did not correlate with the activities of S-phase marker enzymes (i.e., DNA polymerase-α and TK1), which support the cell cycle-independent synthesis of the dCK enzyme. The differences in dCK activity in lymphomas, different leukemias, and solid tumors seems probably correlated to their differentiation stages, with higher levels of dCK in undifferentiated lymphoid and embryonic neural cells.

The normal differentiation pathway is apparently disturbed in children with Down syndrome (DS, trisomy of chromosome 21), who have a 10- to 20-fold increased risk of developing acute lymphoid leukemia and acute myeloid leukemia (AML) compared to children without DS *(19,20)*. However, DS leukemic lymphoblasts and myeloblasts are more sensitive to ara-C toxicity, leading to longer event-free survival rates after treatment compared to children without DS. This may be related to DS leukemic cells containing two to three times higher dCK levels than other leukemic cells *(20)*.

Comparisons have been done among the levels of dCK mRNA, the level of enzyme activity, and dCK protein using dCK-C-terminal peptide antibodies in different cell lines and transplanted tumors *(21,22)*. There was overall a good correlation between these three parameters, except in some cases. These results may be important for future studies in which the level of dCK in patients could be considered as a parameter to obtain individualized chemotherapy with nucleoside analogs, potentially leading to higher efficacy and reduced side effects.

A positive correlation was found between dCK activity and the sensitivity of malignant cells to chemotherapy *(20–23)*, discussed in Section 7. Resistance to nucleoside analogs has been attributed to impaired dCK function caused by various genetic and epigenetic alterations, as summarized below (e.g., point mutations, exon deletions, and alternatively spliced mRNAs). Antisense oligonucleotides and retroviral introduction of hammerhead ribozymes targeted against dCK mRNA have been used and were reported to result in both decreased dCK activity and sensitivity to cytidine analogs *(24)*. Furthermore, full sensitivity to ara-C could be restored by

viral transfer of the dCK complementary DNA (cDNA) into ara-C-resistant cell lines *(25,26)*.

The gene for human dCK is localized to chromosome band 4q13.3–q21.1 and is a 34-kb single copy gene. The true localization of the dCK protein in cells has been unclear. During biochemical isolation procedures, dCK was found in the cytosol, but the enzyme has a nuclear localization signal sequence in the N-terminal region *(27)*. However, endogenous dCK was found only in the cytosol using immunohistochemistry with a dCK peptide antibody, and nuclear localization of dCK was found only in dCK-overexpressing cells *(28)*. The possible role of such a redistribution process of dCK between the cytosol and nuclei remains to be determined.

3. INCREASE OF dCK ACTIVITY BY TREATMENT OF CELLS WITH GENOTOXIC AGENTS: IMPLICATIONS FOR DNA REPAIR AND APOPTOSIS

The sensitivity of malignant cells to deoxyribonucleoside chemotherapeutic drugs is highly influenced by the cellular dCK levels, so that high dCK activity enhances the efficacy of treatment of the cells with nucleoside derivatives. Pretreatment of resistant cells with bryostatin 1 has been shown to exert an adjuvant effect on the sensitivity of the cells to CdA toxicity *(29)*. The mechanism behind this effect has been studied, and it was shown that a 1- to 2-h pretreatment of human lymphocytes with CdA caused a two- to threefold increase in dCK activity not accompanied by any increase in dCK mRNA or protein levels *(30)*. The TK1 or TK2 isoenzyme activities were not increased by this or other treatments of cells with genotoxic agents, so this phenomenon was specific for dCK. During serial dilutions and affinity chromatography, purification the elevated dCK activity was maintained, indicating that feedback regulation by small molecules or allosteric factors is unlikely *(31)*. On the other hand, incubation with λ protein phosphatase decreased the specific activity of dCK in cell extracts, suggesting that reversible phosphorylation of the enzyme might be involved in this process *(31)*. It has also been reported that protein kinase C in vitro phosphorylates and "activates" recombinant dCK *(32)*, but the increase of dCK activity by this mechanism could not be confirmed in human lymphocytes *(33)*.

CdA is an analog of deoxyadenosine that is not deaminated by adenosine deaminase and has excellent clinical activity against hairy cell leukemia and chronic lymphocytic leukemias of B- and T-cell types. CdA is an excellent substrate for dCK in vitro *(34)* as well as in vivo, and its main product, Cl-dAMP, accumulates in human lymphocytes during short-term incubations, during which the activity of dCK increased two to four times *(35)*. The same enhancement of dCK activity occurred on preincubation with a variety

of nonnucleoside genotoxic agents such as aphidicolin *(35)*, etoposide (VP16) *(36,37)*, taxol, and even the G protein modulator sodium fluoride *(38,39)*. γ-Irradiation *(40)* and UV-C irradiation *(41)* also augmented dCK activity in different lymphoid cells. The decrease of dCK activity was observed with protein phosphatase inhibitors *(40)*, again suggesting a regulatory role for reversible protein phosphorylation in the activation process. The sequence of treatment has also been shown to be important, and a synergistic effect was observed with sequential etoposide and cytarabine therapy combinations *(42,43)*.

The effects of nucleoside-5′-thiosulfate (-5′-TS) derivatives of four deoxy- and two ribonucleosides were measured on the activity of the two main salvage dNKs, dCK and TK1, in primary lymphoid cells. Only thymidine-5′-TS (dThd-5′-TS) enhanced the activity of dCK in cells; none of the other nucleoside-5′-TS derivatives had any effect on either the TK or the dCK activities *(44)*.

The activation of dCK can be achieved not only in lymph node cells but also in thymocytes, spleen cells, and peripheral blood lymphocytes. A marked difference in the extent of dCK activation was found between G- and S-phase enriched subpopulations of lymphocytes. In resting lymphocytes, the extent of stimulation was higher than in S-phase cells *(18)*. The activation of dCK was seen also with treatment of cells with the natural nucleoside dAdo, provided that its deamination was abolished by the addition of the adenosine-deaminase inhibitor deoxycoformycine *(45)*. This could be a contributing factor in adenosine deaminase deficiency, which is associated with a dramatic decrease of T lymphocytes and intracellular accumulation of deoxyadenosine 5′-triphosphate (dATP) *(46)*. An imbalance in the nucleotide pools seems to be a primary reason for DNA damage and inhibition of DNA synthesis and repair.

All the treatments described are toxic for cells, disturbing metabolic processes and leading finally to damage of DNA. DNA damage caused by UV-C or γ-irradiation increased the repair of DNA in parallel with the elevation of dCK activity *(40,41)*, and the dATP concentration was also increased in cells *(46)*, which was shown to be involved in apoptosis *(47)*. The damaged DNA triggered rapid hyperphosphorylation and induction of p53, which promotes either DNA repair or apoptosis *(47,48)*. To explore whether p53 is involved in the activation of dCK on genotoxic stress, pifithrin-α, a recently discovered pharmacological inhibitor of p53, was used *(49)*. Treatment of cells with this inhibitor reduced the activation of dCK *(93)*, suggesting a direct or indirect role for p53 in the enzyme activation process *(93)*.

Unexpectedly, the dCK activity was 22-fold higher in CEM cells transfected with the cystathione-β-synthase cDNA (a gene localized to chromosome 21 involved in cysteine biosynthesis from homocystein)

compared to wild type. However, levels of dCK mRNA and protein were not changed in the transfected cells, similar to toxic treatments of cells. Only the dCK enzyme activity was for as-yet-unknown reasons enhanced by transfection of cells with cystathione-β-synthase cDNA *(20)*. The transfection of cells by a cDNA might disturb metabolic processes similarly to other genotoxic treatments, presented before. The final answer was the increase of dCK activity and the increase of the deoxynucleoside salvage and DNA repair.

The special function of the dCyt salvage in the DNA repair was suggested in an early work in which two dCTP pools were shown in lymphocytes *(8)*; this suggestion was also supported in other cells, where functional compartmentation of dCTP pools was shown *(9)*. Thus, the reason for the increase of dCK activity after toxic treatments of cells seems first to be to supply the repair of DNA.

4. PREVENTION OF dCK ACTIVATION BY dCyt AND DEPLETION OF CYTOSOLIC Ca^{2+} IONS

Among nucleoside-monophosphate analogs, only dThd-5′-TS enhanced the activity of dCK in cells; no other nucleoside-5′-TS derivatives had any effect on either dCK or TK activities *(44)*. The activation process of dCK by dThd-5′-TS and other agents can be prevented by simultaneous addition of dCyd; dThd had no effect on the activation process *(44)*.

The counteracting effect of dCyt and not of dThd, preventing the activation of dCK induced by toxic treatments, was surprising. Similar effects of dCyt were observed in totally different systems; for instance, induction of the glycoprotein hormone αa-subunit and placental alkaline phosphatase by butyrate was also inhibited only by dCyt and not by other nucleosides in HeLa cells *(51)*. The treatment with butyrate could be a similar stress signal for the cells as the toxic treatments listed above. The mechanisms of this "protecting" effect of dCyt against the different "stress" treatments leading to increased dCK activity and DNA repair are still unknown. It might be important to supply all four deoxynucleosides by the action of dCK and by the dCyt–dThd nucleotide interconversion pathway, operating mainly in lymphoid cells *(7)* (Figs. 1 and 2).

Protein phosphorylation is involved in the signaling pathway induced by γ-irradiation in the case of the P53R2 RR *(53)*, and it was suggested that the dCK activation is also dependent on protein phosphorylation *(31,32,52,54)*. BAPTA-AM (1,2-*bis* [2-amino-phenoxy] ethane-*N,N,N′,N′*-tetraacetic acid tetrakis [acetoxymethyl ester]), a cell-permeable calcium chelator, *(55,56)* selectively inhibited the activation of dCK in a time- and concentration-dependent manner, but extracellular calcium depletion had no effect on dCK activity *(50)*. Denaturing Western blots of extracts from lymphocytes treated with CdA or other genotoxic agents (i.e., DNA polymerase inhibitor

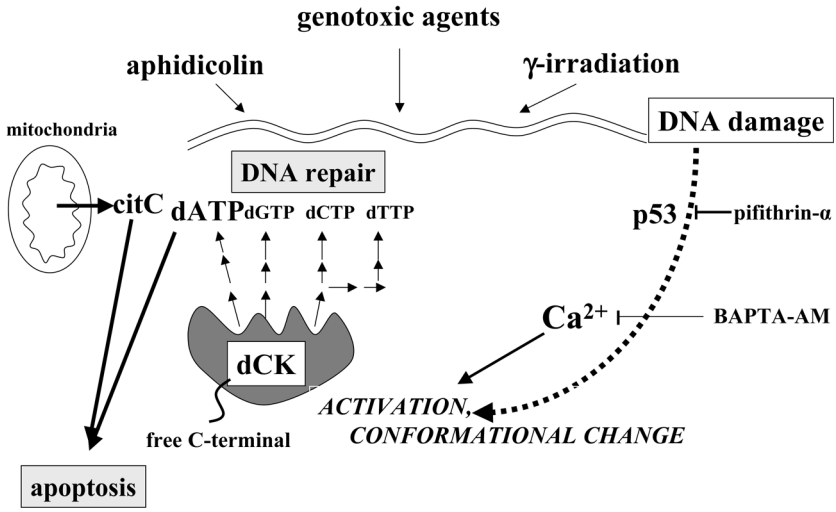

Fig. 5. The mechanism of activation of dCK and induction of apoptosis.

aphidicolin) or with the intracellular calcium chelator BAPTA-AM showed that dCK protein levels were not changed during these treatments *(50)*; the enzyme activity was six to eight times higher in cells treated with genotoxic agents than in cells with low Ca^{2+} ion concentration. However, analysis by Western blotting of native gels surprisingly demonstrated a striking correlation between enzyme activity and the intensity of dCK-specific bands. Apparently, the "high-activity" (presumably modified) form of dCK was more efficiently recognized by the antibody than the "low-activity" dCK form existing at low Ca^{2+} levels in BAPTA-AM-treated cells *(50)*. The exact molecular background for this modification process remains to be clarified.

The molecular mechanism of the toxic treatments inducing increase of dCK activity can be seen in Fig. 5. All toxic treatments (nucleoside analogs, UV or γ-irradiation, etc.) caused DNA damage, which needs DNA repair. The dNTP pools were supplied by the increased dCK activity; the activation of dCK needs Ca^{2+} ions and an intact p53 protein. However, the increased dATP pool is ready to induce the apoptotic machinery if DNA cannot be totally repaired (Fig. 5).

5. DEOXYNUCLEOSIDE ANALOGS ACTIVATED BY dCK

In Chapter 3, Munch-Petersen and Piškur list the pharmacologically most important deoxynucleosides and the anabolizing enzymes responsible for their activation in humans (*see* Chapter 3, Table 2). Here, we shortly

summarize the role of dCK in this process; the background is that, as mentioned, dCK is often the rate-limiting enzyme in the activation of these analogs. L-2′3′-Dideoxy-3′-thiacytidine (lamivudine, 3TC); ara-C; CdA; and 2′,2′-difluorodeoxycytidine (gemcitabine, dFdC) (*1–4, 24, 57,* and *58* and references therein), the first a human immunodeficiency virus drug and the last three valuable anticancer agents, are all substrates for dCK, and they are between 5% and 50% as efficient as dCyt as substrates for the enzyme. Gemcitabine belongs to a new category of anticancer nucleoside analogs that shows activity against solid tumors; other chapters in this book are devoted to describing the use of this drug as well as the key role of dCK as an anabolizing enzyme.

In addition to the analogs mentioned, the antileukemia drug 2-fluoro-arabinofuranosyladenine (fludarabine, F-ara-A) and the antiviral agents 2′,3′-dideoxycytidine (zalcitabine, ddC) and arabinosyladenine (vidarabine, ara-A) are also phosphorylated by dCK *(1–4,57)*. ddC is about 10–20% as efficient as a substrate as dCyt; the two other arabinosyl analogs are less active.

dCK in not selective in the case of the enantiomeric forms of its substrates and in some cases shows preferential phosphorylation of L-nucleosides *(58–63)*. Several L-nucleosides that are substrates for dCK have been developed as antiviral and anticancer drug. Two of these new L-nucleosides; L-FMAU (2′-fluoro-5-methyl-β-L-arabinofuranosyluracil) and L-OddC (β-L-(–)-dioxolane-cytidine), show relatively high activity with dCK. The former analog has broad antiviral activity, and the second shows good promise as an antitumor agent *(62,64)*.

The fact that α-ddC is a more efficient substrate for dCK than β-ddC *(61)* indicates that dCK prefers nucleoside sugars in the S-conformation (C2′-endo-C3′-exo) because α-ddC adopts that conformation preferentially. This has been verified by direct determination of the structure of the nucleoside bound to dCK using nuclear magnetic resonance methods *(65)*. The results are in agreement with molecular modeling studies with herpes simplex virus type 1 TK and conformationally restricted nucleoside analogs *(66)* and should be considered in the future design of nucleoside analogs.

In addition to the analogs briefly described, there are many other nucleosides known to be phosphorylated by dCK. The overall pattern is that dCK accepts both purine and pyrimidines with many modifications in the base, some of which, like 2-Cl or 2-Br adenosine analogs, are better substrates than the natural compounds. Furthermore, many sugar modifications are accepted, and here fluoro-substituted sugars are often excellent substrates, as are arabionsyl analogs. Both L- and D-analogs are accepted, but acyclic nucleoside analogs are very poor substrates for dCK; this is part of the reason for the antiviral properties of the acyclic analogs.

6. STRUCTURE–ACTIVITY RELATIONSHIPS OF dCK

dCK is composed by two identical polypeptides of 261 amino acids *(67)*, and it shows some significant sequence similarity with the herpes simplex type 1 virus TK, as well as about 40% sequence identity to TK2 and dGK. The structure of a dGK-ATP complex was determined in 2000 *(68)*, and in 2003 the structure of dCK in complex with dCyt and ADP-Mg^{2+} was solved *(69)*. Both dGK and dCK show large similarity in structure, and they have six β-sheets and seven or eight α-helices; the N-terminal phosphate-binding loop is in the center of the active site. In dCK, helices 5 and 6 are short; these are one long helix (helix 5) in the case of dGK *(4,68,69)*, but otherwise the folds of the proteins are almost identical, and differences can only be seen in the active site . Both have the 5′-OH group of the nucleoside held in place by hydrogen bonds to a conserved Glu, the amino group of an Arg, and most likely of a water molecule.

The 3′-OH of the deoxyribose interacts with a Glu and a Tyr, and the latter is "overlapping" the 2′-position so that a hydroxy group from a ribonucleotide is prevented from binding with high affinity. The base is bound by hydrogen bonds to a conserved Gln and a Phe. However, the difference between dGK and dCK apparently stems from an altered hydrogen bond network in the active sites, where an Asp residue interacts with an Arg but only in the case of dGK is there a Ser also participating and stabilizing this network. In contrast, in dCK the Ser is substituted by an Ala, and this means that the Arg residue is in a less-extended conformation and allows another position of the Asp, which thus can form a hydrogen bond with the amino group of dCyt. The "same" Arg residue (R104 in dCK and R118 in dGK) is in both enzymes responsible for interacting with the purine base, and it is assumed that in dCK the flexibility in the active site is such that the Arg can also adopt an extended conformation like that found for dGK.

This very elegant explanation for the mechanism behind the structure–activity relationships of dCK and dGK has been supported by the construction of several different mutants in these key residues, both for dCK *(69)* and the homologs *Drosophila* dNK (*4,70*, and Figs. 6 and 7). Overall the understanding of the broad specificity of dCK has made a fundamental advance by the structure determinations of dCK not only in complex with dCyt and ADP-Mg^{2+}, but also in complex with Ara-C-ADP-Mg^{2+} and dFdC-ADP-Mg^{2+} *(69)*. However, still there is no structure of dCK interacting with a phosphate donor or a purine deoxynucleoside, and the structure of the open conformation of dCK is not yet known.

The phosphate donors of dCK most likely bind in the opposite direction compared to the nucleosides, and the β- and γ-phosphates interact with the Lys and Ser residues of the phosphate loop in the N-terminal and two

arginines in the LID region (residues 186–1999) covering the active site *(4,68,69)*. Results regarding the effects of mutations in this region for the specificity of dCK are described next; however, direct structure determinations are needed to clarify the role of the LID region in enzyme catalysis.

A large number of enzyme kinetic studies have been performed with dCK and the reaction kinetics when studied with a wide range of substrate concentrations follows a pattern of negative cooperativity for both the phosphate donor and acceptors, giving Hill coefficients >1 *(1–4,71,72)*. The reaction follows a random bi-bi pathway with ATP; with UTP at low nucleoside concentration, it appears to be ordered with the donor binding first *(70–74)*. This conclusion based on kinetics is difficult to explain in light of the structure of the active site, where the deoxynucleoside is bound in the interior and the donor supposedly binds externally *(4,68,69)*.

Fluorescence quenching, UV difference spectroscopy and near-UV CD spectroscopy demonstrate that substrate binding induces several different conformational changes *(72,74,75)*, with apparently one binding site (or state) for the donors but two binding sites (or states) for the acceptors. These results indicate that the enzyme may exist in different conformational states with different affinities for the substrates, and maybe the open state of the enzyme can bind either substrate alone nonproductively. Only when both bind in the correct subsites simultaneously is a conformational change induced, forming the closed catalytically competent state of dCK.

Feedback inhibitors (e.g., dCTP) act as bisubstrate analogs, binding to both sites in the active cleft, blocking the enzyme *(4,76)*; in case of the *Drosophila* dNK enzyme, the structure of such an enzyme feedback inhibitor complex has been solved *(77)*. It was shown that inorganic tripolyphosphate was a good phosphate donor for dCK when dCyt was the acceptor but not when dAdo was the substrate *(78)*, again indicating that the nature of the donor and acceptor mutually affects its other capacity to participate in the catalytic cycle.

Mouse dCK has a lower capacity to phosphorylate certain deoxynucleosides (e.g., dAdo and ddC) compared with human dCK, which is one reason for that these analogs are less toxic to mice than to humans. The amino acid sequences are 93% identical between mouse and human dCK, but the enzymes show different nucleoside substrate specificity with ATP or UTP used as phosphate donors. The amino acids in the LID region (i.e., in the phosphate donor site) differ in several important positions (particularly Q179R, T184K, H187N) between the mouse and human dCK *(79)*. If these amino acids are changed by mutagenesis, then the specificity of the mutated mouse dCK is indistinguishable from the normal human dCK. Introduction of such a triple mutant (i.e., a humanized form of mouse dCK) into mice may create a better animal model for future nucleoside drug testing.

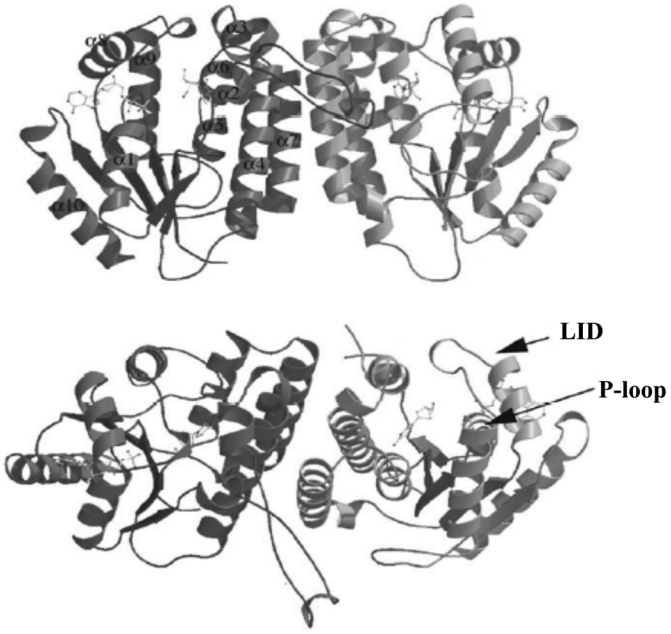

Fig. 6. Comparison of the amino acid sequences of human dCK, dGK, and TK2 and that of the *Drosophila*. Amino acids: identical read, similar: yellow. Structural motives aa:1–10 and β: 1–5 are characteristic for dCK. (From ref. *16*, with permission.)

7. dCK IN CELLS RESISTANT TO TOXIC NUCLEOSIDES

Cell lines lacking dCK are resistant to nucleoside analogs, and dCK deficiency was shown to be a reason for ara-C, dFdC, and CdA resistance (and crossresistance) in cultured cells *(23,80–85)*. However, when leukemic cells were isolated from patients with acute AML who were resistant to ara-C, only in 1 of 16 cases showed dCK deficiency *(86)*. In a separate study of ara-C-resistant AML cells, several shorter dCK mRNA variants were found, and these truncated forms of dCK mRNAs lacked one or several exons *(87)*. However, introduction of these alternatively spliced forms of dCK into leukemic cells did not confer the resistance phenotype to the transfected cells; thus, the role of alternate spliced or truncated forms of dCK mRNA in AML cells *(87)* and in dFdC-resistant ovary cancers cells *(80)* is still unclear.

Three-point mutations leading to inactive dCK have been defined by reconstruction and characterization of the recombinant dCK mutants (i.e., G28E in the P loop, Q156R in α-helix 7, and Y99C in α-helix 4) *(68,81,86)*. In these cases, only the Q156R mutation is in an apparently nonconserved residue, but α-helix 7 is responsible for the subunit interactions. Therefore,

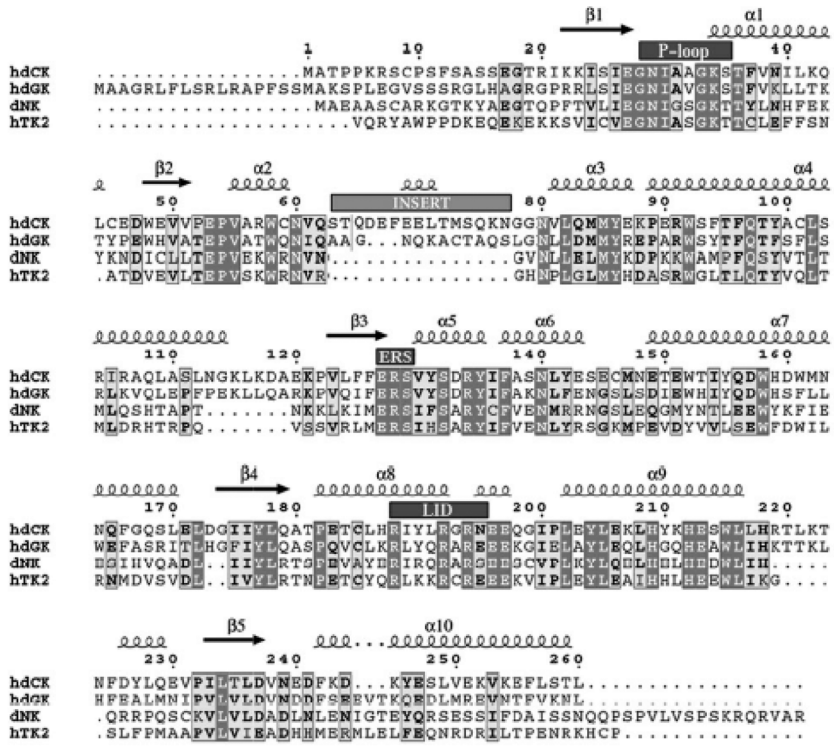

Fig. 7. The homodimer structure of the recombinant dCK. (From ref. *16*, with permission.)

introduction of a charged amino acid may lead to inactivating alterations in the subunit structure of dCK. However, these defined dCK point mutations are only found in a minor fraction of cells resistant to toxic dCK-activated analogs. Apparently, other, more indirect and multiple alteration such as altered splicing and translational and posttranslational mechanisms are involved in the generation in vivo of the resistance phenotype.

In several studies, a correlation between the sensitivity of cells to nucleoside analogs and the level of dCK was observed (e.g., patients responding to CdA had a somewhat higher level of dCK than nonresponders) *(34,88)*. However, the correlation was relatively weak, and in some studies no correlation was observed *(89,90)*. It has been shown that the pretreatment dCK activity or mRNA levels predicted the in vivo sensitivity of transplantable human tumors to treatment with dFdC *(22)*. A significant predictive value of determining the dCK and cNII 5′-nucleotidase levels in AML blast cells at the time of diagnosis has been established in relation to the sensitivity of

treatment of the patients with ara-C *(91)*. Thus, it now seems clear that determinations of dCK levels with highly sensitive and selective methods such as quantitative real-time polymerase chain reaction in the clinical situation is of diagnostic or prognostic value when combined with other parameters, such as the level of catabolic enzymes and other general parameters such as age.

Transfection experiments with dCK gene constructs have shown that higher cellular levels of dCK give higher sensitivity of the cells to cytotoxic nucleosides that serve as substrates for the enzyme *(25,26,92)*. Therefore, dCK gene transfer to tumor cells could be a method to increase the sensitivity for a nucleoside analog. Transfection of glioma cells with vectors containing the dCK gene gave higher sensitivity of the transfected cells to ara-C, and when tested in animal models, it led to tumor regression in response to ara-C chemotherapy *(26)*.

8. CONCLUSIONS

The role of dCK in the supply of DNA precursors is gradually being elucidated, and new knowledge both from enzyme structure and function studies and from studies of deoxynucleoside-resistant cells supports a key function for this enzyme in the deoxynucleoside salvage pathway. The involvement of dCK in liponucleotide metabolism is also obvious even though much remains to be learned about the biological consequences of this pathway. The posttranslational activation of dCK observed after treatment of cells with DNA synthesis inhibitors appears to be a compensatory mechanism induced by metabolic stress signals.

Cells need deoxynucleotides to repair damaged DNA; the increased dCK activity can help supply all four dNTPs, and this may be a unique source of DNA precursors in quiescent cells. The dCK activation process is dependent on the intracellular Ca^{2+} levels and normal function of the p53 protein. The activated form of dCK seems to be an altered stable conformation of the enzyme in which the C-terminal is differently exposed to immunoreagents.

Further studies are needed to define the molecular mechanisms responsible for the activation process, but it is clear that increased understanding in this field may lead to the development of new drug combinations in antitumor or antiviral chemotherapy. The structure of dCK was recently determined, and thus it is now possible to define some of the structure–function relationships of this enzyme and the related expanding dNK enzyme family.

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REFERENCES

1. Arnér ES, Eriksson S. Mammalian deoxyribonucleoside kinases. Pharmacol Ther 1995;67:155–186.
2. Eriksson S, Arnér ES, Spasokoukotskaja T, et al. Prospective and levels of deoxynucleoside kinases in normal and tumor cells: implications for chemotherapy. Adv Enzyme Regul 1994;34:13–45.
3. Eriksson S, Wang L. The role of the cellular deoxynucleoside kinases in activation of nucleoside analogs used in chemotherapy. In: Chu CK, ed. Recent Advances in Nucleosides: Chemistry and Chemotherapy. Elsevier, 2002:455–475.
4. Eriksson S, Munch-Petersen B, Johansson K, Ekhlund H. Structure and function of cellular deoxyribonucleoside kinases. Cell Mol Life Sci 2002;59:1327–1346.
5. Mathews CK. Deoxycytidylate deaminase, human. In: Wiley Encyclopedia of Molecular Medicine. Vol. 5. Wiley, 2002:1327–1346.
6. Gribaudo G, Riera L, Caposio P, Maley F, Landolfo S. Human cytomegalovirus requires cellular deoxycytidylate deaminase for replication in quiescent cells. J Gen Virol 2003;84:1437–1441.
7. Staub M, Spasokukotskaja T, Benczur M, Antoni F. DNA synthesis and nucleoside metabolism in human tonsillar lymphocyte subpopulations. Acta Otolaryngol 1987;454:118–124.
8. Spasokoukotskaja T, Sasvari-Szekely M, Taljanidisz J, Staub M. Compartmentation of dCTP pools disappears after hydroxyurea or araC treatment in lymphocytes. FEBS Lett 1992;297:151–154.
9. Xu YZ, Huang P, Plunkett W. Functional compartmentation of dCTP pools. J Biol Chem 1995;270:631–637.
10. Anglana M, Apiou F, Bensimon A, Debatisse M. Dynamics of DNA replication in mammalian somatic cells: nucleotide pool modulates origin choice and interorigin spacing. Cell 2003;114:385–394.
11. Spasokoukotskaja T, Spyrou G, Staub M. Deoxycytidine is salvaged not only into DNA but also into phospholipid precursors. Biochem Biophys Research Commun 1988;155:923–929.
12. Spasokukotskaja T, Taljanidisz J, Sasvári-Székely M, Staub M. Deoxycytidine is salvaged not only into DNA but also into phospholipid precursors III. dCDP-Diacylglycerol formation in tonsillar lymphocytes. Biochem Biophys Res Commun 1991;174:680–687.
13. Sasvári-Székely M, Spasokoukotskaja T, Staub M. Deoxyribocytidine is salvaged not only into DNA but also into phospholipid precursors IV. Exogenoue deoxycytidine can be used with the same efficacy as (ribo)cytidine for phospholipid activation. Biochem Biophys Res Commun 1993;194:966–723.
14. Hrabák A, Spasokukotskaja T, Temesi A, Staub M. The salvage of deoxycytidine into dCDP-diacylglycerol by macrophages and lymphocytes. Biochem Biophys Res Commun 1993;193:212–219.
15. Martin DP, Wallace TL, Johnson EM Jr. Cytosine arabinoside kills postmitotic neurons in fashion resembling trophic factor deprivation: evidence that deoxycytidine dependent process may be required for nerve growth factor signal transduction. J Neurosci 1990;10:184–193.

16. Nagy N, Magyar T, Spasokoukotskaja T, Virga S, Oláh I, Staub M. The neurotoxicity of 2-Cl-deoxyadanosine can be released by deoxycytidine. Fund Clin Pharmacol 1999;13:46.
17. Taljanidisz J, Spasokukotskaja T, Sasvari-Szekely M, Antoni F, Staub M. Preferential utilisation of deoxycytidine by undifferentiated (peanut positive) tonsillar lymphocytes. Immunol Lett 1987;15:109–115.
18. Horvath L, Sasvari-Szekely M, Spasokoukotskaja T, Antoni F, Staub M. Different utilisation of deoxycytidine and thymidine by tonsillar lymphocyte subpopulations. Immunol Lett 1989;22:161–166.
19. Taub JW, Huang XM, Matherly LH, et al. Expression of chromosome 21 localised genes in acute myeloid leukemia: differences between Down syndrome and non-Down syndrome blast cells and relationship to in vitro sensitivity to cytosine arabinoside and daunorubicin. Blood 1999;94:1393–1400.
20. Taub JW, Huang X, Ge Y, et al. Cystathione-b-synthase cDNA transfection alters the sensitivity and metabolism of 1-β-D-arabinosylcytosine in CCRF-CEM leukemia cells in vitro and in vivo: a model of leukemia in Down syndrome. Cancer Res 2000;60:6421–6426.
21. van der Wilt CL, Kroep JR, Loves WJ, et al. Expression of deoxycytidine kinase in leukaemic cells compared with solid tumour cell lines, liver metastases and normal liver. Eur J Cancer 2003;39:691–697.
22. Kroep JR, Loves WJ, van der Wilt CL, et al. Pretreatment deoxycytidine kinase levels predict in vivo gemcitabine sensitivity. Mol Cancer Ther 2002;1:371–376.
23. Galmarini CM, Mackey IR, Dumontet C. Nucleoside analogues: mechanism of drug resistance and reversal strategies. Leukemia 2001;15:875–890.
24. Beausejour CM, Tremblay G, Momparler RL. Potential of ribozymes against deoxycytidine kinase to confer drug resistance to cytosine nucleoside analogs. Biochem Biophys Res Commun 2000;278:569–575.
25. Stegmann AP, Honders WH, Willemze R, Ruiz van Haperen VW, Landegent JE. Transfection of wild-type deoxycytidine kinase (dCK) cDNA into an AraC- and DAC-resistant rat leukemic cell line of clonal origin fully restores drug sensitivity. Blood 1995;85:1188–1194.
26. Manome Y, Wen PY, Dong Y, et al. Viral vector transduction of the human deoxycytidine kinase cDNA sensitizes glioma cells to the cytotoxic effect of cytosine arabinoside in vitro and in vivo. Nat Med 1996;2:567–573.
27. Johansson M, Brismar S, Karlsson A. Human deoxycytidine kinase is located in the cell nucleus. Proc Natl Acad Sci USA 1997;94:11,941–11,945.
28. Hatzis P, Al-Madhoon AS, Jullig M, Petrakis TG, Eriksson S, Talianidis I. The intracellular localization of deoxycytidine kinase. J Biol Chem 1998;273:30,239–30,243.
29. Mohammad RM, Beck FW, Katato K, Hamdy N, Wall N, Al-Katib A. Potentiation of 2-chlorodeoxyadenosine activity by bryostatin 1 in the resistant chronic lymphocytic leukemia cell line (WSU-CLL): association with increased ratios of dCK/5′-NT and Bax/Bcl-2. Biol Chem 1998;379:1253–1261.
30. Sasvari-Szekely M, Spasokoukotskaja T, Szoke M, et al. Activation of deoxycytidine kinase during inhibition of DNA synthesis by 2-chloro-2′-deoxyadenosine (cladribine) in human lymphocytes. Biochem Pharmacol 1998;56: 1175–1179.
31. Csapo Z, Sasvari-Szekely M, Spasokoukotskaja T, Talianidis I, Eriksson S, Staub M. Activation of deoxycytidine kinase by inhibition of DNA synthesis in human lymphocytes. Biochem Pharmacol 2001;61:191–197.

32. Wang LM, Kucera GL. Deoxycytidine kinase is phosphorylated in vitro by protein kinase C alpha. Biochim Biophys Acta 1994;1224:161–167.
33. Spasokoukotskaja T, Csapo Z, Sasvari-Szekely M, et al. Effect of phosphorylation on deoxycytidine kinase activity. Adv Exp Med Biol 2000;486:281–285.
34. Arnér ES, Spasokoukotskaja T, Juliusson G, Eriksson S. Phosphorylation of 2-chlorodeoxyadenosine (CdA) in extracts of peripheral blood mononuclear cells of leukemic patients. Br J Haematol 1994;87:715–718.
35. Sasvari-Szekely M, Piroth Z, Kazimierczuk Z, Staub M. A novel effect of the new antileukemic drug, 2-chloro-2′-deoxyadenosine, in human lymphocytes. Biochem Biophys Res Commun 1994;203:1378–1384.
36. Spasokoukotskaja T, Sasvari-Szekely M, Keszler G, Albertioni F, Eriksson S, Staub M. Treatment of normal and malignant cells with nucleoside analogues and etoposide enhances deoxycytidine kinase activity. Eur J Cancer 1999;35:1862–1867.
37. Ooi K, Ohkube T, Higashigawa M, Kawasaki H, Sakurai M. Increased deoxycytidine kinase activity by etoposide in L1210 murine leukemic cells. Biol Pharm Bull 1996;19:1382–1383.
38. Staub M, Csapó Zs, Spasokoukotskaja T, Sasvári-Székely M. Deoxycytidine kinase can be also potentiated by the G-protein activator NaF in cells. Adv Exp Med Biol 1998;431:425–428.
39. Csapo Z, Sasvari-Szekely M, Spasokoukotskaja T, Staub M. Modulation of human deoxycytidine kinase activity as a response to cellular stress induced by NaF. Acta Biochim Pol 2001;48:251–256.
40. Csapo Zs, Keszler G, Safrany G, et al. Activation of deoxycytidine kinase by gamma-irradiation and inactivation by hyperosmotic shock in human lymphocytes. Biochem Pharmacol 2003;65:2031–2039.
41. Van den Neste E, Smal C, Delacauw A, et al. Activation of deoxycytidine kinase by UV-C irradiation in chronic lymphatic leukemia B-lymphocytes. Biochem Pharmacol 2003;65:573–580.
42. Kreder CN, van Bree C, Peters GJ, Loves PJ, Haveman J. Enhanced levels of deoxycytidine kinase and thymidine kinase 1 and 2 after pulsed low dose rate irradiation as an adaptive response to radiation. Oncol Rep 2002;9:141–144.
43. Ohkubo T, Higashigava M, Kavasaki H, et al. Synergistic interaction between etoposide and 1-β-arabinofuranosylcytosine. Adv Exp Med Biol 1989;253B:355–362.
44. Keszler G, Szikla K, Kazimierczuk Z, Spasokoukotskaja T, Sasvari-Szekely M, Staub M. Selective activation of deoxycytidine kinase by thymidine-5′-thiosulphate and release by deoxycytidine in human lymphocytes. Biochem Pharmacol 2003;65:563–571.
45. Hershfield MS, Mitchell BS. Immunodeficiency diseases caused by adenosine deaminase deficiency and purine nucleotide phosphorylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The Metabolic and Molecular Bases of Inherited Disease. 7th ed. McGraw-Hill Health Professions, New York, NY, vol. 2. 1995:1725–1769.
46. Keszler G, Spasokoukotskaja T, Csapo Zs, Virga S, Staub M, Sasvari-Szekely M. Selective increase of dATP pools upon activation of deoxycytidine kinase in lymphocytes: implications in apoptosis. Nucleosides Nucleotides Nucleic Acids 2004; 23:1335–1342.
47. Genini D, Budihardjo L, Plunkett W, et al. Nucleotide requirements for the in vitro activation of the apoptosis protein-activating factor-1-mediated caspase pathway. J Biol Chem 2000;275:29–34.

48. Oren M. Decision making by p53: life, death and cancer. Cell Death Differ 2003;10:431–442.
49. Komarov PG, Komarova EA, Kondratov RV, et al. A chemical inhibitor of p53 that protects mice from the side effects of cancer therapy. Science 1999;285:1733–1737.
50. Keszler G, Spasokoukotskaja T, Csapo Zs, et al. Activation of deoxycytidine kinase in lymphocytes is calcium dependent and involves a conformational change detectable by native immunostaining. Biochem Pharmacol 2004;67;947–955.
51. Tamura RN, Cox GS. Effect of pyrimidine deoxynucleosides and sodium butirate on expression of the glycoprotein hormone a-subunit and placental alkaline phosphatase in HeLa cells. Biochem Biophys Acta 1988;968:151–159.
52. Achanta G, Pelicano H, Feng L, Plunkett W, Huang P. Interaction of p53 and DNA-PK in response to nucleoside analogues: potential roles as a sensor complex for DNA damage. Cancer Res 2001;61:8723–8729.
53. Yamaguchi T, Matsuda K, Sagiya Y, et al. p53R2-dependent pathway for DNA synthesis in p53 regulated cell cycle checkpoint. Cancer Res 2001;61:8256–8261.
54. Smal C, Vertommen D, Bertrand L, et al. Identification of in vivo phosphorylation sites on human deoxycytidine kinase. Biol Chem 2006;281:4887–4893.
55. Tombal B, Denmeade SR, Gillis JM, Isaacs JT. A supramicromolar elevation of intracellular free calcium is consistently required to induce the execution phase of apoptosis. Cell Death Differ 2002;9:561–573.
56. Dieter P, Fitzke E, Duyster J. BAPTA induces a decrease of intracellular free calcium and a translocation and inactivation of protein kinase C in macrophages. Biol Chem Hoppe Seyler 1993;374:171–174.
57. Plunkett W, Gandhi V. Purine and pyrimidine nucleoside analogs. Cancer Chemother Biol Response Modif 2001;19:21–45.
58. Gumina G, Chong Y, Choo H, Song GY, Chu CK. L-Nucleosides: antiviral activity and molecular mechanism. Curr Top Med Chem 2002;2:1065–1086.
59. Chang CN, Skalski V, Zhou JH, Cheng YC. Biochemical pharmacology of (+)- and (–)-2′,3′-dideoxy-3′-thiacytidine as anti-hepatitis B virus agents. J Biol Chem 1992;267:22,414–22,420.
60. Maury G. The enantioselectivity of enzymes involved in current antiviral therapy using nucleoside analogues: a new strategy? Antivir Chem Chemother 2000;11: 165–189.
61. Wang J, Choudhury D, Chattopadhyaya J, Eriksson S. Stereoisomeric selectivity of human deoxyribonucleoside kinases. Biochemistry 1999;38:16,993–16,999.
62. Grove KL, Cheng YC. Uptake and metabolism of the new anticancer compound β-L-(–)-dioxolane-cytidine in human prostate carcinoma DU-145 cells. Cancer Res 1996;56:4187–4191.
63. Verri A, Focher F, Priori G, et al. Lack of enantiospecificity of human 2′-deoxycytidine kinase: relevance for the activation of β-L-deoxycytidine analogs as antineoplastic and antiviral agents. Mol Pharmacol 1997;51:132–138.
64. Liu SH, Grove KL, Cheng YC. Unique metabolism of a novel antiviral L-nucleoside analog, 2′-fluoro-5-methyl-β-L-arabinofuranosyluracil: a substrate for both thymidine kinase and deoxycytidine kinase. Antimicrob Agents Chemother 1998;42: 833–839.
65. Maltseva T, Usova E, Eriksson S, Milecki J, Foldesi A, Chattopadhayaya J. An NMR conformational study of the complex of $^{13}C/^{2}H$ double labeled 2′-deoxynucleoside and deoxycytidine kinase. J C S Perkin Trans 2000;2:199–2207.

66. Prota A, Vogt J, Pilger B, et al. Kinetics and crystal structure of the wild-type and the engineered Y101F mutant of Herpes simplex virus type 1 thymidine kinase interacting with (North)-methanocarba-thymidine. Biochemistry 2000;39:9597–9603.
67. Chottiner EG, Shewach DS, Datta NS, et al. Cloning and expression of human deoxycytidine kinase cDNA. Proc Natl Acad Sci USA 1991;88:1531–1535.
68. Johansson K, Ramaswamy S, Ljungcrantz C, et al. Structural basis for substrate specificities of cellular deoxyribonucleoside kinases [erratum in: Nat Struct Biol 2001;8:818–819]. Nat Struct Biol 2001;8:616–620.
69. Sabini E, Ort S, Monnerjahn C, Konrad M, Lavie A. Structure of human dCK suggests strategies to improve anticancer and antiviral therapy. Nat Struct Biol 2003;10:513–519.
70. Knecht W, Sandrini MP, Johansson K, Eklund H, Munch-Petersen B, Piskur J. A few amino acid substitutions can convert deoxyribonucleoside kinase specificity from pyrimidines to purines. EMBO J 2002;21:1873–1880.
71. Ives DH, Durham JP. Deoxycytidine kinase. 3. Kinetics and allosteric regulation of the calf thymus enzyme. J Biol Chem 1970;245:2285–2294.
72. Kierdaszuk B, Rigler R, Eriksson S. Binding of substrates to human deoxycytidine kinase studied with ligand-dependent quenching of enzyme intrinsic fluorescence. Biochemistry 1993;32:699–707.
73. Hughes TL, Hahn TM, Reynolds KK, Shewach DS. Kinetic analysis of human deoxycytidine kinase with the true phosphate donor uridine triphosphate. Biochemistry 1997;36:7540–7547.
74. Turk B, Awad R, Usova EV, Björk I, Eriksson S. A pre-steady state kinetic analysis of substrate binding to human recombinant deoxycytidine kinase: a model for nucleoside kinases. Biochemistry 1999;38:8555–8561.
75. Mani RS, Usova EV, Eriksson S, Cass CE. Hydrodynamic and spectroscopic studies of substrate binding to human recombinant deoxycytidine kinase. Nucleosides Nucleotides Nucleic Acids 2003;22:175–192.
76. Ikeda S, Chakravarty R, Ives DH. Multisubstrate analogs for deoxynucleoside kinases. Triphosphate end products and synthetic bisubstrate analogs exhibit identical modes of binding and are useful probes for distinguishing kinetic mechanisms. J Biol Chem 1986;261:15,836–15,843.
77. Mikkelsen NE, Johansson K, Karlsson A, et al. Structural basis for feedback inhibition of the deoxyribonucleoside salvage pathway: studies of the *Drosophila* deoxyribonucleoside kinase. Biochemistry 2003;42:5706–5712.
78. Krawiec K, Kierdaszuk B, Shugar D. Inorganic tripolyphosphate (PPP(i)) as a phosphate donor for human deoxyribonucleoside kinases. Biochem Biophys Res Commun 2003;301:192–197.
79. Usova EV, Eriksson S. Identification of residues involved in the substrate specificity of human and murine dCK. Biochem Pharmacol 2002;64:1559–1567.
80. Ruiz van Haperen VW, Veerman G, Eriksson S, et al. Development and molecular characterization of a 2′,2′-difluorodeoxycytidine-resistant variant of the human ovarian carcinoma cell line A2780. Cancer Res 1994;54:4138–4143.
81. Owens JK, Shewach DS, Ullman B, Mitchell BS. Resistance to 1-β-D-arabinofuranosylcytosine in human T-lymphoblasts mediated by mutations within the deoxycytidine kinase gene. Cancer Res 1992;52:2389–2393.
82. Dumontet C, Fabianowska-Majewska K, Mantincic D, et al. Related common resistance mechanisms to deoxynucleoside analogues in variants of the human erythroleukaemic line K562. Br J Haematol 1999;106:78–85.

83. Lotfi K, Månsson E, Spasokoukotskaja T, et al. Biochemical pharmacology and resistance to 2-chloro-2′-arabino-fluoro-2′-deoxyadenosine, a novel analogue of cladrabine in human leukemic cells. Clin Cancer Res 1999;5:2438–2444.
84. Mansson E, Spasokoukotskaja T, Sallstrom J, Eriksson S, Albertioni F. Molecular and biochemical mechanisms of fludarabine and cladribine resistance in a human promyelocytic cell line. Cancer Res 1999;59:5956–5963.
85. Mansson E, Flordal E, Liliemark J, et al. Down-regulation of deoxycytidine kinase in human leukemic cell lines resistant to cladribine and clofarabine and increased ribonucleotide reductase activity contributes to fludarabine resistance. Biochem Pharmacol 2003;65:237–247.
86. Flasshove M, Strumberg D, Ayscue L, et al. Structural analysis of the deoxycytidine kinase gene in patients with acute myeloid leukemia and resistance to cytosine arabinoside. Leukemia 1994;8:780–785.
87. Veuger M, Honders M, Landegent J, Willemze R, Barge R. High incidence of alternatively spliced forms of deoxycytidine kinase. Blood 2000;96:1517–1524.
88. Kawasaki H, Caricia CJ, Carson DA. Quantitative immunoassay of human deoxycytidine kinase in malignant cell. Anal Biochem 1992;207:193–196.
89. Leiby JM, Snider KM, Kraut EH, Metz EN, Malspeis L, Grever MR. Phase II trial of 9-β-D-arabinofuranosyl-2-fluoroadenine 5′-monophosphate in non-Hodgkin's lymphoma: prospective comparison of response with deoxycytidine kinase activity. Cancer Res 1987;47:2719–2722.
90. Abertioni F, Lindemalm S, Reichlova V, et al. Pharmacokinetics of cladribine in plasma and its 5′-mono and triphosphate in leucemic cells in patients with chronic lymphocytic leukemia. Clin Cancer Res 1998;4:653–658.
91. Galmarini CM, Thomas X, Graham K, et al. Deoxycytidine kinase and cN-II nucleotidase expression in blast cells predict survival in acute myeloid leukaemia patients treated with cytarabine. Br J Haematol 2003;122:53–60.
92. Hapke DM, Stegmann APA, Mitchell BS. Retroviral transfer of deoxycytidine kinase into tumor cells lines enhances nucleoside toxicity. Cancer Res 1996;56:2343–2347.
93. Keszler G, Virga Sz, Spasokuokotskaja T, Bauer PI, Sasvari-Székely M, Staub M. Activation of deoxycytidine kinase by deoxyadenosine: implication in deoxyadenosine mediated cytotoxicity. Arch Biochem Biophys 2005;436:69–77.

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