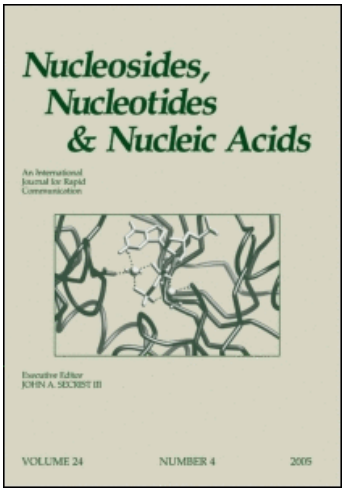

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Selective Increase of dATP Pools upon Activation of Deoxycytidine Kinase in Lymphocytes: Implications in Apoptosis

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ABSTRACT

Stimulation of the activity of deoxycytidine kinase (dCK), the principal deoxynucleoside salvage enzyme, has been recently considered as a protective cellular response to a wide range of agents interfering with DNA repair and apoptosis. In light of this, the potential contribution of dCK activation to apoptosis induction—presumably by supplying dATP or its analogues for the apoptosome formation—deserves consideration. Two-hour exposure of human tonsillar lymphocytes to 2-chloro-deoxyadenosine (CdA) led to a two-fold activation of dCK. This activation process was inhibited by pifithrin-α, a potent inhibitor of p53. When the dNTP pools were determined, both deoxypyrimidine triphosphate and dGTP pools were reduced after the treatments, while dATP levels elevated by 62%, 77% and 50% in the CdA, aphidicolin and etoposide-treated cells, respectively. We assume that dCK activation elicited by cellular damage might be a proapoptotic factor in terms of generating dATP well before the release of cytochrome c and deoxyguanosine kinase from mitochondria.

Key Words: Deoxycytidine kinase; dNTP pools; Apoptosis; p53; Pifithrin-α.

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INTRODUCTION

In resting cells, where the activity of ribonucleotide reductase is low, deoxyribonucleotide precursors for DNA repair are supplied mainly by the salvage pathways. Deoxycytidine kinase (dCK) is a key enzyme of the salvage pathway (for a recent review see Ref. [1]). As a consequence of its broad substrate specifity, dCK efficiently phosphorylates several deoxynucleoside analogues used in antiviral and anticancer therapy, such as 2-chlorodeoxyadenosine (Cladribine, CdA) or arabinosylcytosine (araC)[1] reflecting the high impact of dCK activity on the chemotherapeutic efficacy of deoxynucleoside analogues. In many cell lines, dCK activity shows a good correlation with the cytotoxicity of several drugs, such as gemcitabine.[2] On the other hand, resistant cell lines might have either normal[3] or low[4] dCK activity, showing the importance of other factors that also influence the cytotoxicity of nucleoside analogues.[5]

Elevated enzyme activity of dCK was shown after in vitro CdA treatment of human lymphocytes previously in our laboratory.[6] Activation of dCK was also achieved by direct inhibition of DNA synthesis, pointing to the importance of any disturbances in DNA metabolism that may trigger dCK stimulation.[7] Recently, γ-irradiation of lymphocytes was shown as an activator of the enzyme under conditions where the double strand breaks were completely repaired in a short time.[8] Regarding the mechanism of activation, a post-translational modification was suggested, as the amount of the dCK protein was unchanged under all the above-mentioned conditions.[6–8] Furthermore, protein phosphatase treatment of the cell extracts abolished the activity of dCK, pointing to the possible role of reversible protein phosphorylation in the activation process.[7]

Elevated dCK activity was also found in other cell lines, as the consequence of etoposide treatment[9] as well as UV-[10] or γ-irradiation.[11] Based on these data one could hypothesize that many chemo- and radiotherapeutic agents, exerting their effect through DNA damage, can induce dCK activation which in turn would supply the DNA repair processes with deoxyribonucleotide precursors. On the other hand, it is remarkable that prolonged treatments with these DNA-damaging agents will eventually elicit apoptosis that is the basis of their therapeutic effect.[12–14] Programmed cell death is executed through activation of the p53-initiated signalling pathway upon DNA damage and/or—at least in case of nucleoside analogues—through a direct effect on the mitochondria that is mediated by transient cytoplasmic Ca^{2+} surges.[5,15] Cytochrome c release from the mitochondria is followed by the assembly of the apoptosome which is a dATP-dependent process. Cytochrome c release is accompanied by the efflux of mitochondrial deoxyguanosine kinase into the cytosol which might be involved in the production of dATP.[16] It has been shown that dATP-analogues can supplant the natural deoxynucleotide in the apoptosome formation.[17]

The aim of this study was to explore the potential role of deoxycytidine kinase activation in apoptosis. We present evidence that inhibition of p53 decreases the stimulatory effect of CdA on dCK activity. Also, activation of dCK by several agents selectively augments the intracellular dATP pool that is implicated in the formation of the apoptosome.

MATERIALS AND METHODS

Primary cultures of human tonsillar lymphocytes were incubated in the absence or presence of the indicated drugs for two hours. Enzyme activity was determined in the freeze-thaw cellular extracts using ^{3}HdC as substrate as described previously.[6–8] For dNTP pool assays, cells were extracted with methanol and the pools were selectively determined by the method of Sherman and Fyfe,[18] using synthetic oligonucleotide template primers and Klenow DNA polymerase fragment.

Statistical analysis was performed using the Student *t* test.

RESULTS AND DISCUSSION

Inactivation of p53 reduces the enzyme activity of deoxycytidine kinase p53 performs a critical role in cell cycle control and in the maintenance of the integrity of the genome.[19] DNA damage caused by UV- or gamma-irradiation or by nucleoside analogues triggers the rapid hyperphosphorylation and induction of p53 that promotes either DNA repair or apoptosis.[19,20] To explore whether p53 is involved in the up-regulation of the enzyme activity of dCK upon genotoxic stress, pifithrin-alpha (PFT-α), a recently discovered pharmacological inhibitor of p53 was used.[21] Parallel cultures of human lymphocytes were treated with increasing concentrations of PFT-α alone or in combination with 2 μM CdA and deoxycytidine kinase activities were subsequently measured in the cell extracts.

Figure 1 shows that PFT-α decreased CdA-stimulated dCK levels in a dose-dependent fashion. Inhibition of the enzyme occurred even at low concentrations of the drug (from 198% to 157% at 5 μM concentration). On the other hand, a transient

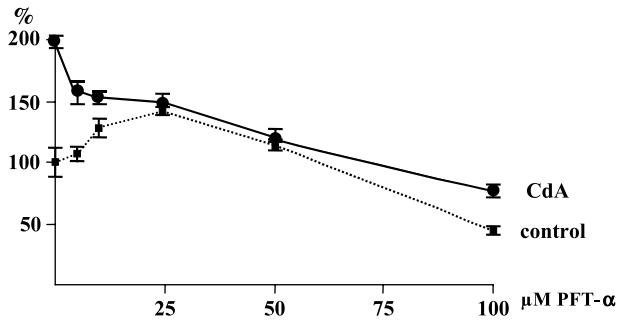

Figure 1. Inhibition of p53 results in decreased deoxycytidine kinase activity. Parallel cultures of human tonsillar lymphocytes were incubated with increasing concentrations of PFT-α (range: 1–100 μM) in the presence (solid line) or in the absence (dotted line) of 2 μM CdA for 2 hours at 37°C. dCK enzyme activities were determined from the crude extracts as described, and plotted as percentages of the untreated control. Control activity (100%) was 4.77 ± 0.40 pmol dCMP/10^6 cells/min. Activity values are the means of two parallels in two independent experiments; error bars represent the standard deviations.

potentiation of the non-stimulated enzyme activity was observed in the 5–25 μM range followed by a gradual suppression well below the control activity at higher concentrations of PFT-α (44% at 100 μM). Cell viability was not affected by the drug (data not shown).

p53 is a transcription factor while dCK is a cytoplasmic protein[22] although it was found in the nucleus upon overexpression.[23] Moreover, dCK is constantly expressed in cells and its activation does not occur at the transcriptional level.[6] At first sight, these facts argue against the potential regulation of dCK activity by p53. However, there are examples proving that p53 can play regulatory roles via protein–protein interactions in the cytoplasm. For instance, p53 physically interacts with the p53R2 and hRRM2 subunits of ribonucleotide reductase and this interaction is abolished when cells are UV-irradiated. Subsequently, the two subunits enter the nucleus to provide dNTPs for DNA repair. Since dCK might perform very similar functions as ribonucleotide reductase and it also has the ability to enter the nucleus due to its nuclear localization signal,[22] potential interactions between p53 and dCK are well worth further investigations.

Selective Increase of the dATP Pool upon dCK Activation

All four deoxyribonucleoside triphosphate pools were determined in cells exposed to different treatments as indicated in Table 1. dTTP pools were generally decreased irrespective of the applied treatments that either suppressed (hyperosmotic shock with sorbitol) or augmented (etoposide, aphidicolin and chlorodeoxyadenosine) the activity of deoxycytidine kinase in cells, while dGTP pools decreased significantly upon aphidicolin and CdA treatments. dCTP pools were only decreased in response to treatments that heighten dCK activity, whereas they seem generally unaffected by those that suppress dCK activity. On the other hand, dATP pools were sustained when the enzyme was downregulated but showed 50%, 77% and 62% increases when dCK was activated with etoposide, APC or CdA, respectively. Since we found increased dATP pools and elevated dCK activities as the results of treatments with three different genotoxic agents and also upon gamma-irradiation of cells (T. Spasokoukotskaja, unpublished observations), it is reasonable to assume a causal relationship between these events.

Severe combined immunodeficiency syndrome (SCID) is the clinical manifestation of adenosine deaminase deficiency where accumulated dATP plays a causal role in the apoptotic death of both T and B lymphocytes. However, according to our best knowledge, no elevations were found in the dCK activities of the lymphocytes of these patients. This suggests that dATP accumulation was due to the stimulated dCK activity in our experiment and not the other way round (Table 1). We assume that activated dCK phosphorylates deoxyadenosine preferentially out of its three endogenous substrates. The biological relevance of this bias might imply the contribution of deoxycytidine kinase to apoptosis when the cell is irreparably damaged. Increased dATP levels promote the formation of the apoptosome and the activation of caspases thereafter.

Jullig and Eriksson proposed that the released deoxyguanosine kinase might play a similar role in the phosphorylation of deoxyadenosine.[16] Importantly, deoxycytidine kinase activation can be observed very early during the stress response to proapoptotic agents[6–8] and dATP pool increases were found within two hours of incubation,

Table 1. Cellular deoxynucleoside triphosphate pool sizes during activation or inactivation of deoxycytidine kinase.

	Control	Sorbitol	NaCl	Etoposide	APC	CdA
dCK activity	8.0 ± 0.1***	3.4 ± 0.2***	2.1 ± 0.2***	20.0 ± 0.1***	18 ± 0.1***	19.7 ± 0.7***
	(100%)	(42%)	(26%)	(250%)	(225%)	(246%)
dNTP pools						
dCTP	4.6 ± 0.4	5.7 ± 0.5*	4.3 ± 0.3	2.6 ± 0.4***	3.3 ± 0.7**	3.0 ± 0.7**
	(100%)	(124%)	(94%)	(57%)	(71%)	(65%)
dTTP	3.2 ± 0.1	2.2 ± 0.9**	2.7 ± 0.7	1.1 ± 0.1***	2.3 ± 0.6*	2.0 ± 0.3***
	(100%)	(68%)	(84%)	(33%)	(73%)	(61%)
dGTP	3.5 ± 0.8	3.2 ± 0.7	3.0 ± 0.1	3.0 ± 0.1	2.4 ± 0.3**	2.5 ± 0.3**
	(100%)	(90%)	(85%)	(86%)	(70%)	(73%)
dATP	1.8 ± 0.5	1.9 ± 0.7	1.9 ± 0.8	**2.7 ± 0.6****	**3.2 ± 0.5****	**2.9 ± 0.6****
	(100%)	(106%)	(106%)	**(150%)**	**(177%)**	**(162%)**

dCK activities are expressed as pmol/10^6 cells/min, deoxynucleotide pools as pmol/10^6 cells. Means and standard deviations were calculated from three independent experiments. Differences between the control and treated samples were considered statistically significant as follows: *$p < 0.10$; **$p < 0.05$; ***$p < 0.01$.

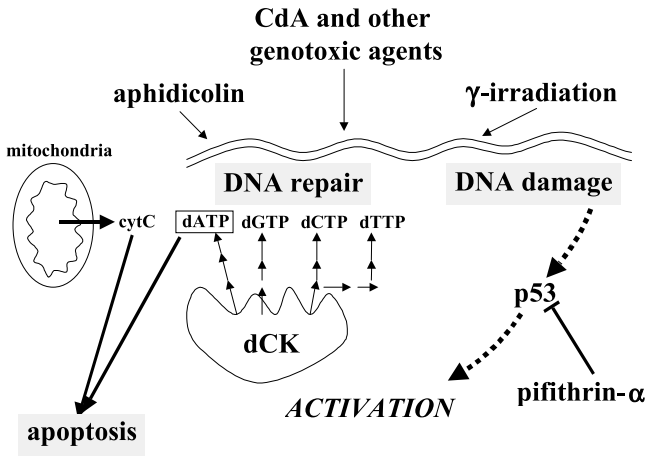

Figure 2. Our model for the dual function played by dCK in DNA repair and apoptosis.

suggesting that activated dCK might accumulate dATP well before the release of cytochrome c and deoxyguanosine kinase from the mitochondria (Fig. 2).

In conclusion, we propose that dCK activation following DNA damage might perform a dual function either by serving the survival of cells by producing dNTPs for DNA repair or by providing dATP for the apoptosome. However, details of the regulation of the enzyme activation still remain to be studied.

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