FEBS Letters 588 (2014) 727–732

FEBS Letters

journal homepage: www.FEBSLetters.org

Protein phosphatase 2A regulates deoxycytidine kinase activity *via* Ser-74 dephosphorylation

Rachid Amsailale [a], Maxime Beyaert [a], Caroline Smal [a], Veerle Janssens [b], Eric Van Den Neste [a,c], Françoise Bontemps [a,*]

[a] *de Duve Institute & Université Catholique de Louvain, B-1200 Brussels, Belgium*
[b] *Laboratory of Protein Phosphorylation and Proteomics, Department of Cellular and Molecular Medicine, University of Leuven, B-3000 Leuven, Belgium*
[c] *Department of Hematology, Cliniques Universitaires Saint-Luc & Université Catholique de Louvain, B-1200 Brussels, Belgium*

ARTICLE INFO

Article history:
Received 22 October 2013
Revised 10 December 2013
Accepted 8 January 2014
Available online 23 January 2014

Edited by Zhijie Chang

Keywords:
Deoxycytidine kinase
Nucleoside analog
Ser-74 phosphorylation
Protein phosphatase
Ser/Thr protein phosphatase 2A

ABSTRACT

Deoxycytidine kinase (dCK) is a critical enzyme for activation of anticancer nucleoside analogs. Its activity is controlled *via* Ser-74 phosphorylation. Here, we investigated which Ser/Thr phosphatase dephosphorylates Ser-74. In cells, the PP1/PP2A inhibitor okadaic acid increased both dCK activity and Ser-74 phosphorylation at concentrations reported to specifically target PP2A. In line with this, purified PP2A, but not PP1, dephosphorylated recombinant pSer-74-dCK. In cell lysates, the Ser-74-dCK phosphatase activity was found to be latent, Mn^{2+}-activated, responsive to PP2A inhibitors, and diminished after PP2A-immunodepletion. Use of siRNAs allowed concluding definitively that PP2A constitutively dephosphorylates dCK in cells and negatively regulates its activity.

1. Introduction

Deoxycytidine kinase (dCK) converts deoxycytidine, deoxyguanosine and deoxyadenosine into their monophosphate form with ATP or UTP as phosphoryl donor. This reaction is the first and rate-limiting step of the deoxyribonucleoside salvage pathway that supplies cells with precursors of DNA as an alternative to *de novo* synthesis [1]. In addition, dCK phosphorylates and thereby activates several anticancer and antiviral nucleoside analogs, such as fludarabine, cytarabine, gemcitabine, zalcitabine and lamivudine [2,3].

Given the essential physiological and pharmacological role of dCK, identification of the mechanisms that regulate its activity is of particular interest. The first mechanism reported to control dCK activity was retro-inhibition by dCTP, although its physiological relevance is questionable [4–6]. Thereafter, several studies [7–10] suggested that dCK activity could be regulated by post-translational modification, which was definitively confirmed by our group. We established that dCK is a phosphoprotein containing at least four phosphorylation sites: Thr-3, Ser-11, Ser-15 and Ser-74, the latter being the major phosphorylated residue and the only one to play a role in the regulation of dCK activity [11]. Furthermore, we found that increase in dCK activity induced by genotoxic treatments, such as etoposide, aphidicolin, UV-irradiation, was correlated to an increase of Ser-74 phosphorylation, whereas decrease of its activity induced by osmotic stress was related to Ser-74 dephosphorylation [11,12]. Insight into the mechanism by which Ser-74 phosphorylation modifies dCK activity has recently been provided through structural studies of Hazra et al. [13], who propose that phosphorylation of Ser-74 favors the adoption by dCK of an open conformation more conducive for binding of nucleoside and release of the nucleoside monophosphate product.

The finding of the phosphorylation of dCK at Ser-74 raised the question of which protein kinase(s) and phosphatase(s) directly phosphorylate(s) or dephosphorylate(s) this residue. Casein kinase 1δ was shown to be able to phosphorylate dCK on Ser-74 *in vitro*, but would not be responsible for its *in vivo* phosphorylation [14]. In contrast, the DNA-damage-response kinase ATM (ataxia telangiectasia mutated) was found to phosphorylate Ser-74 *in vitro,* but also *in vivo,* which was also associated with a role of dCK in the

Abbreviations: dCK, deoxycytidine kinase; pSer-74-dCK, dCK phosphorylated at Ser-74; ATM, ataxia telangiectasia mutated; PP, Ser/Thr phosphatase; PP1, Ser/Thr protein phosphatase 1; PP1c, catalytic subunit of PP1; PP2A, Ser/Thr protein phosphatase 2A; PP2Ac, catalytic subunit of PP2A; Fos, Fostriecin; OA, okadaic acid; siRNA, small interfering RNA; HEK, human embryonic kidney

* Corresponding author. Address: de Duve Institute, Université Catholique de Louvain, Avenue Hippocrate, 75, 1200 Brussels, Belgium. Fax: +32 2764 7598.
E-mail address: francoise.bontemps@uclouvain.be (F. Bontemps).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2014.01.016

regulation of the G2/M checkpoint [15]. Concerning the protein phosphatase (PP) that dephosphorylates Ser-74 in intact cells, its responsiveness to okadaic acid (OA) has been established. Indeed, this PP inhibitor increased dCK activity [16] and Ser-74 phosphorylation [14] in cultured cells.

Ser/Thr specific protein phosphatases comprise three structurally unrelated superfamilies and have been implicated in the regulation of many cell events [17,18]. The vast majority of Ser and Thr dephosphorylations in eukaryotic cells are catalyzed by members of the PPP (phosphoprotein phosphatases) and PPM (protein phosphatase metal) families. PPMs are Mg^{2+}/Mn^{2+}-dependent monomeric enzymes with PP2C phosphatases as the major representatives and are insensitive to OA inhibition. PPPs comprise the large families of multimeric Ser/Thr protein phosphatase 1 (PP1), Ser/Thr protein phosphatase 2A (PP2A) and PP2B (calcineurin) phosphatases, as well as the more recently identified PP4, PP5, PP6 and PP7 phosphatases, which are expressed at relatively low abundance. PP4 and PP6 are sometimes referred to as PP2A-like phosphatases due to their high structural homology with PP2A and similar sensitivities to pharmacological phosphatase inhibitors. Among Ser/Thr phosphatases, PP2A is potently inhibited by OA in the low nanomolar range, while PP1 is around 100 times less sensitive. In contrast, PP4 and PP6 have similar IC_{50} values for OA than PP2A [19]. In the present study, we sought to determine which OA-sensitive PP is responsible for Ser-74 dephosphorylation and hence is involved in the regulation of dCK activity.

2. Material and methods

2.1. Materials

Dulbecco's Modified Eagle Medium (DMEM), ultraglutamine, fetal calf serum (FCS) and penicillin–streptomycin were purchased from Lonza. RPMI-1640 and all tissue culture reagents were from Gibco/Invitrogen. [5-^{3}H]-deoxycytidine (30 Ci/mmol) was from Moravek Biochemicals. OA was from Enzo Life Sciences. Anti-phospho-Ser-74 and anti-dCK antibodies were generated as described previously [11]. Anti-poly(His) monoclonal antibody, protein A Sepharose beads and NAP-5 columns were from GE Healthcare. Anti-PP1c (sc-7482), anti-PP2Ac (sc-166034) antibodies and fostriecin (Fos) were from Santa Cruz Biotechnology. Fluorescent secondary antibodies IRDye®680 or IRDye®800 and Odyssey blocking buffer were from Li-COR Biosciences. Catalytic subunit of PP2A (specific activity: 10 U/mg), purified from beef heart according to Cohen et al. [20], and GST-tagged catalytic subunit of PP1 (specific activity: 8 U/mg), purified after overexpression in *Escherichia coli*, were kindly provided by Prof. M. Rider (de Duve Institute, Brussels, Belgium). Other chemicals, materials and reagents were from Sigma–Aldrich, Merck Biosciences or Bio-Rad Laboratories.

2.2. Cell culture and incubation

CCRF-CEM cells and HEK293T cells that stably express human dCK were cultured as described [11,14]. EBV-transformed lymphoblasts GM0536 (ATM+/+) and GM1526 (ATM−/−), purchased from The NIGMS human Mutant Cell Repository, were maintained in RPMI-1640 with Glutamax supplemented with 15% heat-inactivated FCS. OA was dissolved in DMSO and equal amounts of DMSO were added in control cells.

2.3. Preparation of cell lysates and dCK assay

CCRF-CEM, GM0536, GM1526 or HEK293T cell extracts were prepared as previously described [11,16]. dCK activity was measured with a radiochemical assay as described in [16] using 10 μM [5-^{3}H]deoxycytidine and 5 mM ATP as substrates and 30–100 μg of cellular protein or 0.05–0.1 μg of recombinant dCK. The protein content of samples was measured by the method of Bradford [21], with BSA as a standard.

2.4. Protein phosphatase treatment of purified recombinant dCK

Recombinant dCK (0.5 μg) overexpressed in HEK293T cells and purified as reported previously [11] was incubated at 30 °C in 50 μl of a reaction mixture containing 50 mM Tris–HCl, pH 7.6, 2 mM dithiothreitol and 2 mM $MnCl_2$ in the absence or in the presence of 0.2 U of purified catalytic subunit of PP1 (PP1c) or catalytic subunit of PP2A (PP2Ac). After 40 min of incubation, aliquots (0.1 μg of protein) were taken for dCK assay and western blot analysis.

2.5. Ser-74-dCK phosphatase assay in cell lysates

For analysis of dCK dephosphorylation at Ser-74 and dCK inactivation in cell lysates, cell extracts were prepared in a phosphatase assay buffer (50 mM Tris–HCl, pH 7.6, 5 mM dithiothreitol, 0.5% Nonidet P-40, 0.1 mM EDTA, 20% glycerol, 0.5 mM p-toluenesulfonyl fluoride, 5 mM benzamidine, and 5 μg/ml leupeptin and antipain). After centrifugation, supernatants were filtered on NAP-5 columns and 300 μg of filtered protein were then incubated at 37 °C for 90 min, as such or in the presence of 0.5 μg of purified recombinant dCK [11]. The reaction was started by the addition of Mn^{2+} at the indicated concentration. At time zero and after 90 min of incubation, aliquots were taken for analysis of dCK phosphorylation at Ser-74 and activity.

2.6. PP2A immunoprecipitation

For PP2A immunoprecipitation, protein A Sepharose beads (60 μl) were incubated with 9 μg of anti-PP2Ac antibody on a rocker for 1 h at 4 °C. The beads were then washed twice with the phosphatase assay buffer and incubated overnight with 900 μg of protein extract at 4 °C. After centrifugation, the supernatant was used for Ser-74-dCK phosphatase analysis as described above.

2.7. RNA interference

HEK293T cells stably expressing dCK were plated in 10 cm-dishes (0.6×10^6 cells/dish) and transfected on the following day using negative control small interfering RNA (siRNA) (non-targeting pool: # D-001810-10, from Dharmacon), 50 nM PP2Ac siRNA (directed against the PP2Ac alpha isoform: # L-003598-010005, from Dharmacon) or 50 nM pan PP1c siRNA (# sc-43545, from Santa Cruz Biotechnology) and 20 μl Lipofectamin 2000 (Invitrogen), according to the manufacturer's instructions. After 72 h, the cells were washed with cold PBS and cell extracts were prepared as described in [16]. After centrifugation, supernatants were used for dCK activity assay and western blot analysis.

2.8. Immunoblot analysis

Aliquots containing 40–100 μg of cell protein or 0.05–0.1 μg of recombinant dCK were subjected to SDS–PAGE electrophoresis in 12% (w/v) polyacrylamide gels and transferred to low fluorescence PVDF immobilon-FL membranes (Millipore). After transfer, PVDF membranes were blocked for 1 h at room temperature in Odyssey blocking buffer and then probed overnight with either anti-phospho-Ser-74 (1/1000), anti-poly(His) (1/4000), anti-dCK (1/500), anti-PP1c (1/1000), anti-PP2Ac (1/1000), or anti-β-actin (1/10,000). After washing in PBS-T (Tween 0.1%), the membranes were incubated for 1 h at room temperature with the appropriate fluorescent secondary antibody IRDye®680 or IRDye®800

(Li-COR) diluted (1/10,000) in Odyssey blocking buffer containing 0.1% Tween and 0.1% SDS. After washing, the membranes were scanned with the Odyssey Infrared Imaging System (Li-COR). Representative immunoblots are shown. When indicated, fluorescence intensities have been used to quantify dCK phosphorylation or expression [22].

2.9. Statistical analysis

dCK activities are the means ± S.E.M. of at least three independent experiments. Significance was analyzed by paired Student's *t*-tests. Changes were considered significantly different when $P < 0.05$.

3. Results and discussion

Our previous findings that dCK activity [16] and dCK phosphorylation at Ser-74 [14] were augmented in intact cells after treatment with 500 nM OA, a cell-permeable PP1/PP2A inhibitor, and the fact that purified PP2A inactivated dCK in cell lysates [16] led us to postulate that PP2A was involved in the *in vivo* regulation of dCK activity. Though OA inhibits both PP1 and PP2A, it has substantial preference *in vitro* for PP2A (IC_{50} = 0.1 nM) over PP1 (IC_{50} = 10 nM) [19]. Its selectivity for PP2A has also been demonstrated *in vivo* in intact cells in which 100 nM OA has been shown to inhibit PP2A without affecting PP1 activity [23]. Therefore, we sought to examine thoroughly the dose–response effect of OA on both dCK phosphorylation at Ser-74 and dCK activity. Analyses were performed after a 4 h-incubation in CCRF-CEM cells in which phosphorylation of dCK at Ser-74 can be easily detected by western blot [11]. Increase in Ser-74 phosphorylation (Fig. 1A) and in dCK activity (Fig. 1B) was already noticed at 25 nM OA, while maximal Ser-74 phosphorylation level and dCK activity were reached at 100 nM. These results suggested that PP2A or a PP2A-like enzyme, rather than PP1, was involved in the control of Ser-74 phosphorylation.

In cells, increase of Ser-74 phosphorylation can result from inhibition of a dCK phosphatase or from activation of a dCK kinase. Nowadays, ATM is the only protein kinase known to phosphorylate dCK at Ser-74 *in vivo* [15]. To verify that the increase of Ser-74 phosphorylation induced by OA was not due to the indirect activation of ATM, we compared the effect of OA in ATM proficient (GM0536) and deficient (GM1526) lymphoblastoid cell lines. We found that OA induced similar dCK activation in both cell lines, either at 100 (not shown) or at 500 nM (Fig. 2A and B). These results indicate that increase in Ser-74 phosphorylation induced by OA cannot be explained by ATM activation, which is consistent with the finding that OA, though inducing autophosphorylation of ATM on Ser-1981, does not increase its kinase activity [24]. Interestingly, these data also indicate that a kinase different from ATM can phosphorylate dCK at Ser-74 at least in basal conditions, which deserves further investigations.

To corroborate the hypothesis that PP2A rather than PP1 is involved in Ser-74 dephosphorylation, we compared the ability of purified catalytic subunits of PP1 (PP1c) and PP2A (PP2Ac) to dephosphorylate Ser-74-dCK. Recombinant dCK overexpressed in HEK293T cells, which is highly phosphorylated at Ser-74 [11], was used as substrate. Incubation of the latter with PP1c induced neither Ser-74 dephosphorylation (Fig. 3A) nor decrease of its activity (Fig. 3B) in comparison with the control condition, whereas PP2Ac used at the same specific activity as PP1c decreased Ser-74 phosphorylation (Fig. 3A) by 70.9 ± 3.9% (n = 3) and dCK activity (Fig. 3B) by 75.2 ± 7.1% (n = 5). We verified that PP1c was active by checking its capacity to dephosphorylate histone H1 ^{32}P-prelabeled by protein kinase A as described in [19] (not shown). We conclude that PP2Ac, but not PP1c, dephosphorylates dCK at Ser-74 *in vitro*.

The next step was to analyze Ser-74-dCK phosphatase activity from cell lysates. For this purpose, CCRF-CEM cell extracts were prepared in a buffer containing no phosphatase inhibitors and then gel-filtered to remove small molecular weight molecules. First, we examined the ability of a cell lysate to dephosphorylate the recombinant dCK. Quite unexpectedly, we found that neither the phosphorylation of recombinant dCK at Ser-74 (Fig. 4A) nor

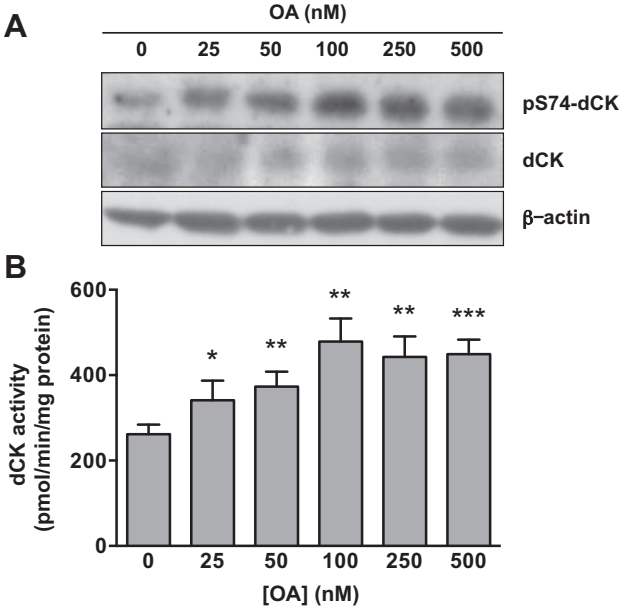

Fig. 1. Dose–response effects of OA on Ser-74-dCK phosphorylation and activity. CCRF-CEM cells were incubated for 4 h with increasing concentrations of OA. Cell lysates were analyzed for (A) dCK phosphorylation at Ser-74 and (B) dCK activity (mean ± S.E.M. of 5 independent experiments). Significance relative to the absence of OA: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

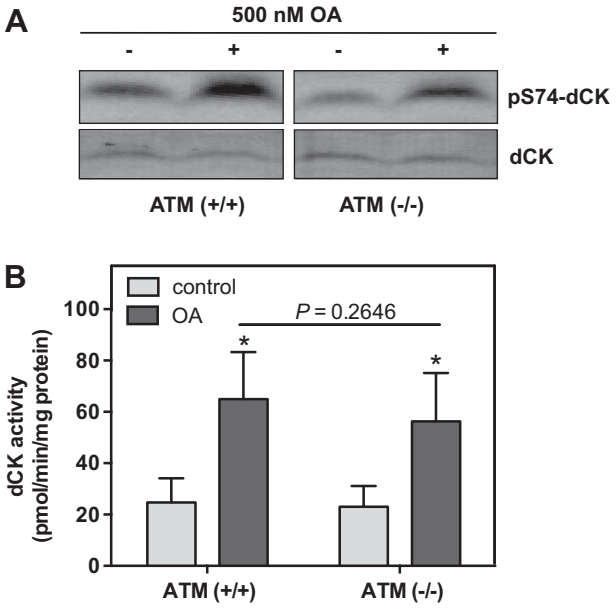

Fig. 2. Comparison of Ser-74 phosphorylation and dCK activation induced by OA in GM0536 (ATM+/+) and GM1526 (ATM−/−) cells. Cells were incubated for 4 h in the absence or the presence of 500 nM OA. Cell lysates were analyzed for (A) dCK phosphorylation at Ser-74 and (B) dCK activity (mean ± S.E.M. of 4 independent experiments). Significance relative to the absence of OA: $^{*}P < 0.05$. Increase of dCK activity induced by OA in ATM (+/+) cells was 2.86 ± 0.37-fold in comparison with 2.5 ± 1.18-fold in ATM (−/−) cells.

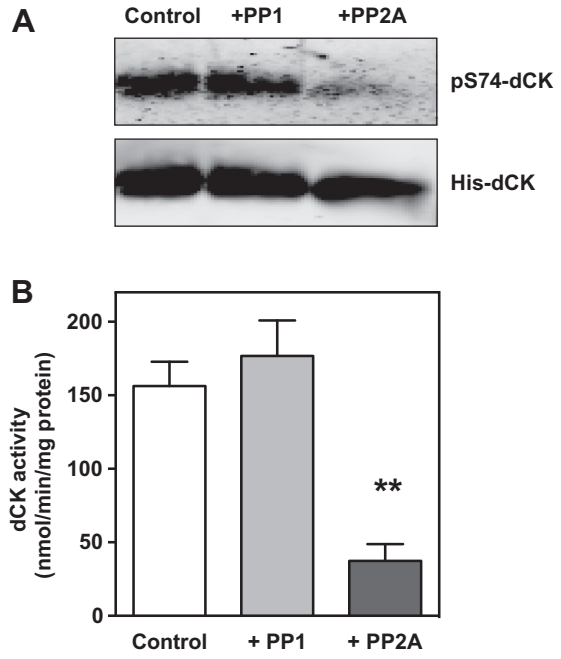

Fig. 3. PP2Ac, but not PP1c, dephosphorylates dCK at Ser-74 *in vitro*. Recombinant dCK, was incubated at 30 °C in the absence or in the presence of purified PP1c or PP2Ac, as described in the Methods. (A) dCK phosphorylation at Ser-74 and (B) dCK activity (mean ± S.E.M. of 5 independent experiments) were measured after 40 min of treatment. Significance relative to the control condition: $^{**}P < 0.01$.

its activity (Fig. 4B) were significantly modified by a 90 min-incubation at 37 °C in the presence of the cell lysate, unless Mn^{2+} was added in the assay mixture at a concentration exceeding 0.1 mM. Similar results were obtained with unfiltered cell lysates (not shown), confirming that Ser-74-dCK phosphatase requires Mn^{2+} to be active. Analysis of the effect of other divalent metal showed that Fe^{2+} or Mg^{2+} could replace Mn^{2+} albeit with a lower efficiency (not shown). Following these experiments, 2 mM Mn^{2+} was included in all *in vitro* Ser-74-dCK phosphatase assays. To verify whether this Mn^{2+}-activated Ser-74-dCK phosphatase could be PP2A, we analyzed whether PP2A inhibitors could inhibit it. We found that OA completely prevented dephosphorylation of the recombinant dCK (Fig. 5A) by the cell lysate and strongly reduced its inactivation (Fig. 5B). This allows to exclude the possibility that Ser-74-dCK phosphatase is a type 2C phosphatase (PP2C), which is characterized by dependence on divalent cations, but insensitivity to OA [25,26]. Fostriecin, a highly selective inhibitor of PP2A [27], also clearly reduced the Mn^{2+}-dependent Ser-74-dCK phosphatase activity from cell lysates (Fig. 5A and B).

Although it is generally accepted that PP2A is not dependent on Mn^{2+} or other divalent cations for activity, metal ions are required in the catalysis of PPP phosphatases through the activation of a water molecule for the dephosphorylation reaction [17]. In addition, some studies reported that PP2A can exist in a latent form, which can be activated *in vitro* by treatment with Mn^{2+} [28–31]. Inactivation of PP2A, which has especially been observed after prolonged storage but also under other conditions, was explained by the loss of metal ions from its catalytic site. The Mn^{2+}-independent PP2Ac has indeed been reported to be a Zn^{2+}- and Fe^{2+}-metalloenzyme [30]. In our hands, the Mn^{2+}-dependency of Ser-74-dCK phosphatase activity was not correlated to the storage and was not due to gel-filtration, but was present as soon as cell lysates were prepared.

To further confirm that Ser-74-dCK phosphatase is PP2A, we compared Ser-74-dCK phosphatase activity of CCRF-CEM cell lysates from which PP2A had been, or not, removed by immunoprecipitation. As observed with recombinant dCK, dephosphorylation (Fig. 6A) and inactivation (Fig. 6B) of endogenous dCK in whole cell lysates were strongly inhibited by OA and fostriecin. Dephosphorylation and inactivation of dCK were also reduced following immunodepletion of PP2A, showing that Ser-74-dCK phosphatase

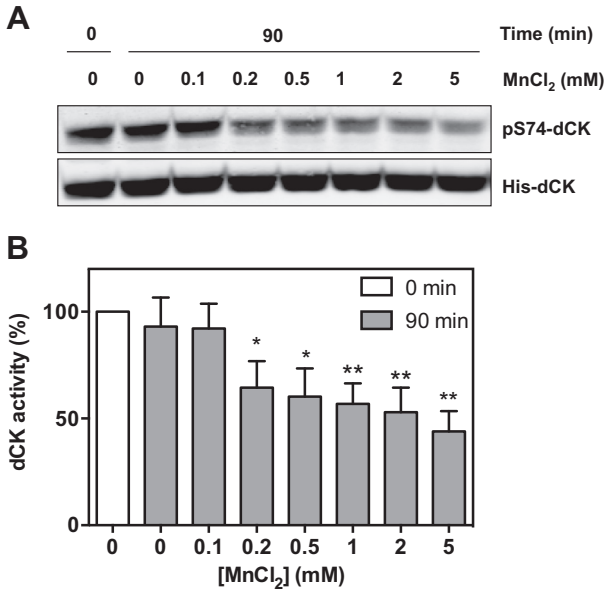

Fig. 4. Mn^{2+} activates Ser-74-dCK phosphatase activity in cell lysates. Recombinant dCK was incubated at 37 °C in the presence of 300 μg of CCRF-CEM cell lysate protein, in the absence or in the presence of $MnCl_2$ at increasing concentrations. (A) Phosphorylation of dCK at Ser-74 and (B) dCK activity (mean ± S.E.M. of 3 independent experiments) were analyzed before (0 min) and after 90 min of incubation. Activity of dCK at 0 min was 197 ± 35 nmol/min/mg protein. Significance relative to the absence of $MnCl_2$: $^{*}P < 0.05$; $^{**}P < 0.01$.

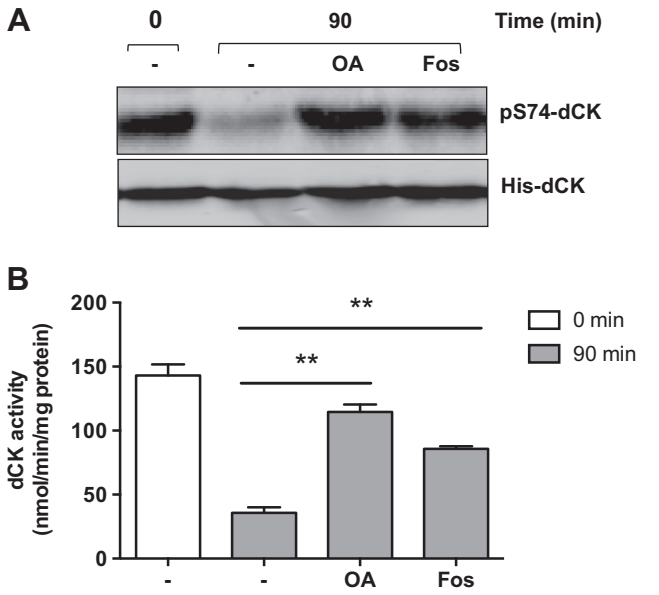

Fig. 5. Ser-74-dCK phosphatase from cell lysate is sensitive to PP2A inhibitors. Recombinant dCK was incubated at 37 °C in the presence of 300 μg of CCRF-CEM cell lysate protein, in the absence or in the presence of 500 nM OA or 500 nM fostriecin (Fos). Mn^{2+} was present at 2 mM. (A) Phosphorylation of dCK at Ser-74 and (B) dCK activity (mean ± S.E.M. of 3 independent experiments) were analyzed before (0 min) and after 90 min of incubation. Significance relative to the absence of PP2A inhibitors: $^{**}P < 0.01$.

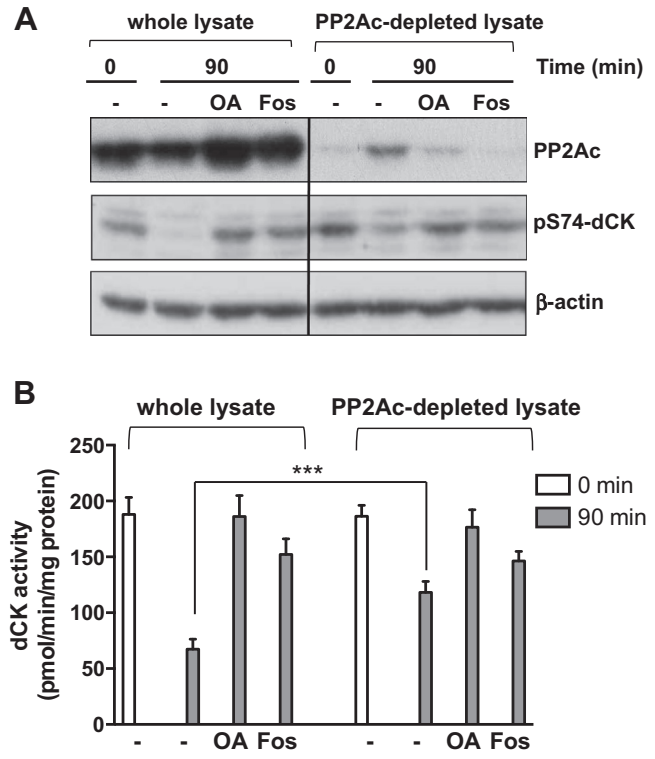

Fig. 6. Effect of PP2Ac immunodepletion on Ser-74-dCK phosphatase activity. CCRF-CEM cell lysates were submitted, or not, to PP2Ac immunoprecipitation and then incubated at 37 °C in the absence or in the presence of 500 nM OA or 1000 nM fostriecin (Fos). Mn^{2+} was present at 2 mM. (A) PP2Ac level, phosphorylation of dCK at Ser-74 and (B) dCK activity (mean ± S.E.M. of 3 independent experiments) were analyzed before (0 min) and after 90 min of incubation. Significance relative to whole cell lysate: $^{***}P < 0.001$.

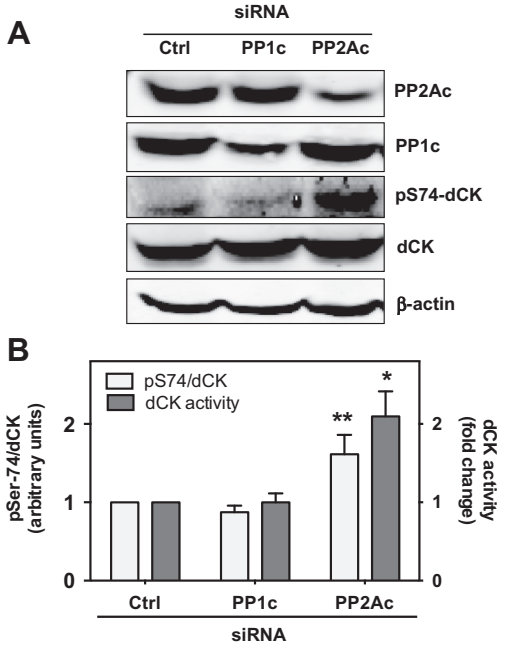

Fig. 7. Effect of PP1c or PP2Ac silencing on Ser-74-dCK phosphorylation and activity in HEK293T cells stably expressing dCK. Cells were transfected with 50 nM control siRNA, PP1c siRNA or PP2Ac siRNA. Cell lysates were analyzed 72 h after transfection for (A) PP2Ac, PP1c, pSer-74-dCK, dCK, β-actin levels and (B) dCK activity (mean ± S.E.M. of 4 independent experiments). In (B) are also shown the pSer-74-dCK/dCK ratios calculated after densitometric quantification of immunoblots (mean ± S.E.M. of 4 independent experiments). Significance relative to control siRNA: $^{*}P < 0.05$; $^{**}P < 0.01$.

activity was diminished in the PP2A-immunodepleted lysates. Addition of OA or fostriecin to PP2A-immunodepleted cell lysates inhibited more completely the dephosphorylation of dCK at Ser-74 and its inactivation. This indicates that Ser-74-dCK phosphatase activity was not completely eliminated by PP2A immunodepletion, which could be explained by residual PP2Ac presence in the supernatant (Fig. 6A). In contrast to what was observed after PP2A immunodepletion, PP1 immunodepletion did not influence the dephosphorylation of Ser-74-dCK and the inactivation of dCK in CCRF-CEM cell lysates (not shown).

To definitively prove that Ser-74-dCK is a physiological substrate for PP2A and not for PP1, we used siRNA to down-regulate the expression of the catalytic subunit of PP2A or PP1. As previously described, we used HEK293T cells stably expressing dCK as experimental model for siRNA transfection [14]. Analysis of PP2Ac and PP1c by immunoblotting showed that after a 72 h-transfection, PP2Ac and PP1c siRNA decreased PP2Ac and PP1c protein levels by 73.3 ± 1.8 and 82.9 ± 5.5% ($n = 4$), respectively. Down-regulation of PP2Ac was accompanied by a significant increase of both dCK phosphorylation at Ser-74 and dCK activity, whereas down-regulation of PP1c did not influence any of them (Fig. 7A and B). These results demonstrate that PP2A is a physiological protein phosphatase for dCK phosphorylated at Ser-74 (pSer-74-dCK).

In conclusion, the present study shows that PP2A is a negative regulator of dCK activity in cells and so adds new information about the signaling pathway that controls dCK activity *in vivo*. Use of PP2A inhibitors could thus be considered as a potential strategy to enhance dCK activity and hence the activation and clinical efficacy of anticancer nucleoside analogs that are substrates of dCK, especially as PP2A inhibitors may be, under certain conditions, promising therapeutic drugs for the treatment of cancer [32–34].

Acknowledgements

This work was supported by Grants from the Belgian Fonds National de la Recherche Scientifique (Télévie, FRSM, FRIA), by the Interuniversity Attraction Poles Program-Belgian Science Policy, the Center of excellence DIANE programme of the Région Walonne, and by private foundations: Jacques Goor and Salus Sanguinis.

References

[1] Arner, E.S. and Eriksson, S. (1995) Mammalian deoxyribonucleoside kinases. Pharmacol. Ther. 67, 155–186.
[2] Van Rompay, A.R., Johansson, M. and Karlsson, A. (2003) Substrate specificity and phosphorylation of antiviral and anticancer nucleoside analogues by human deoxyribonucleoside kinases and ribonucleoside kinases. Pharmacol. Ther. 100, 119–139.
[3] Galmarini, C.M., Mackey, J.R. and Dumontet, C. (2002) Nucleoside analogues and nucleobases in cancer treatment. LANCET Oncol. 3, 415–424.
[4] Sarup, J.C., Johnson, M.A., Verhoef, V. and Fridland, A. (1989) Regulation of purine deoxynucleoside phosphorylation by deoxycytidine kinase from human leukemic blast cells. Biochem. Pharmacol. 38, 2601–2607.
[5] Datta, N.S., Shewach, D.S., Hurley, M.C., Mitchell, B.S. and Fox, I.H. (1989) Human T-lymphoblast deoxycytidine kinase: purification and properties. Biochemistry 28, 114–123.
[6] Amsailale, R., Van Den Neste, E., Arts, A., Starczewska, E., Bontemps, F. and Smal, C. (2012) Phosphorylation of deoxycytidine kinase on Ser-74: impact on kinetic properties and nucleoside analog activation in cancer cells. Biochem. Pharmacol. 84, 43–51.
[7] Sasvari-Szekely, M., Spasokoukotskaja, T., Szoke, M., Csapo, Z., Turi, A., Szanto, I., et al. (1998) Activation of deoxycytidine kinase during inhibition of DNA synthesis by 2-chloro-2′-deoxyadenosine (Cladribine) in human lymphocytes. Biochem. Pharmacol. 56, 1175–1179.
[8] Spasokoukotskaja, T., Sasvari-Szekely, M., Keszler, G., Albertioni, F., Eriksson, S. and Staub, M. (1999) Treatment of normal and malignant cells with nucleoside analogues and etoposide enhances deoxycytidine kinase activity. Eur. J. Cancer 35, 1862–1867.

[9] Csapo, Z., Keszler, G., Safrany, G., Spasokoukotskaja, T., Talianidis, I., Staub, M., et al. (2003) Activation of deoxycytidine kinase by gamma-irradiation and inactivation by hyperosmotic shock in human lymphocytes. Biochem. Pharmacol. 65, 2031–2039.
[10] Keszler, G., Spasokoukotskaja, T., Csapo, Z., Talianidis, I., Eriksson, S., Staub, M., et al. (2004) Activation of deoxycytidine kinase in lymphocytes is calcium dependent and involves a conformational change detectable by native immunostaining. Biochem. Pharmacol. 67, 947–955.
[11] Smal, C., Vertommen, D., Bertrand, L., Ntamashimikiro, S., Rider, M.H., Van Den Neste, E., et al. (2006) Identification of in vivo phosphorylation sites on human deoxycytidine kinase. Role of Ser-74 in the control of enzyme activity. J. Biol. Chem. 281, 4887–4893.
[12] Smal, C., Van Den Neste, E., Maerevoet, M., Poire, X., Theate, I. and Bontemps, F. (2007) Positive regulation of deoxycytidine kinase activity by phosphorylation of Ser-74 in B-cell chronic lymphocytic leukaemia lymphocytes. Cancer Lett. 253, 68–73.
[13] Hazra, S., Szewczak, A., Ort, S., Konrad, M. and Lavie, A. (2011) Post-translational phosphorylation of serine 74 of human deoxycytidine kinase favors the enzyme adopting the open conformation making it competent for nucleoside binding and release. Biochemistry 50, 2870–2880.
[14] Smal, C., Vertommen, D., Amsailale, R., Arts, A., Degand, H., Morsomme, P., et al. (2010) Casein kinase 1delta activates human recombinant deoxycytidine kinase by Ser-74 phosphorylation, but is not involved in the in vivo regulation of its activity. Arch. Biochem. Biophys. 502, 44–52.
[15] Yang, C., Lee, M., Hao, J., Cui, X., Guo, X., Smal, C., et al. (2012) Deoxycytidine kinase regulates the G2/M checkpoint through interaction with cyclin-dependent kinase 1 in response to DNA damage. Nucleic Acids Res. 40, 9621–9632.
[16] Smal, C., Cardoen, S., Bertrand, L., Delacauw, A., Ferrant, A., Van den Berghe, G., et al. (2004) Activation of deoxycytidine kinase by protein kinase inhibitors and okadaic acid in leukemic cells. Biochem. Pharmacol. 68, 95–103.
[17] Shi, Y. (2009) Serine/threonine phosphatases: mechanism through structure. Cell 139, 468–484.
[18] Brautigan, D.L. (2013) Protein Ser/Thr phosphatases – the ugly ducklings of cell signalling. FEBS J. 280, 324–345.
[19] Swingle, M., Ni, L. and Honkanen, R.E. (2007) Small-molecule inhibitors of ser/thr protein phosphatases: specificity, use and common forms of abuse. Methods Mol. Biol. 365, 23–38.
[20] Cohen, P., Alemany, S., Hemmings, B.A., Resink, T.J., Stralfors, P. and Tung, H.Y. (1988) Protein phosphatase-1 and protein phosphatase-2A from rabbit skeletal muscle. Methods Enzymol. 159, 390–408.
[21] Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
[22] Smal, C., Ntamashimikiro, S., Arts, A., Van Den Neste, E. and Bontemps, F. (2010) Influence of phosphorylation of THR-3, SER-11, and SER-15 on deoxycytidine kinase activity and stability. Nucleosides, Nucleotides Nucleic Acids 29, 404–407.
[23] Favre, B., Turowski, P. and Hemmings, B.A. (1997) Differential inhibition and posttranslational modification of protein phosphatase 1 and 2A in MCF7 cells treated with calyculin-A, okadaic acid, and tautomycin. J. Biol. Chem. 272, 13856–13863.
[24] Goodarzi, A.A., Jonnalagadda, J.C., Douglas, P., Young, D., Ye, R., Moorhead, G.B., et al. (2004) Autophosphorylation of ataxia-telangiectasia mutated is regulated by protein phosphatase 2A. EMBO J. 23, 4451–4461.
[25] Cohen, P. (1989) The structure and regulation of protein phosphatases. Annu. Rev. Biochem. 58, 453–508.
[26] Bialojan, C. and Takai, A. (1988) Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics. Biochem. J. 256, 283–290.
[27] Walsh, A.H., Cheng, A. and Honkanen, R.E. (1997) Fostriecin, an antitumor antibiotic with inhibitory activity against serine/threonine protein phosphatases types 1 (PP1) and 2A (PP2A), is highly selective for PP2A. FEBS Lett. 416, 230–234.
[28] Cai, L., Chu, Y., Wilson, S.E. and Schlender, K.K. (1995) A metal-dependent form of protein phosphatase 2A. Biochem. Biophys. Res. Commun. 208, 274–279.
[29] Nishito, Y., Usui, H., Tanabe, O., Shimizu, M. and Takeda, M. (1999) Interconversion of Mn(2+)-dependent and -independent protein phosphatase 2A from human erythrocytes: role of Zn(2+) and Fe(2+) in protein phosphatase 2A. J. Biochem. 126, 632–638.
[30] Nishito, Y., Usui, H., Shinzawa-Itoh, K., Inoue, R., Tanabe, O., Nagase, T., et al. (1999) Direct metal analyses of Mn2+-dependent and -independent protein phosphatase 2A from human erythrocytes detect zinc and iron only in the Mn2+-independent one. FEBS Lett. 447, 29–33.
[31] Longin, S., Jordens, J., Martens, E., Stevens, I., Janssens, V., Rondelez, E., et al. (2004) An inactive protein phosphatase 2A population is associated with methylesterase and can be re-activated by the phosphotyrosyl phosphatase activator. Biochem. J. 380, 111–119.
[32] McConnell, J.L. and Wadzinski, B.E. (2009) Targeting protein serine/threonine phosphatases for drug development. Mol. Pharmacol. 75, 1249–1261.
[33] Kalev, P. and Sablina, A.A. (2011) Protein phosphatase 2A as a potential target for anticancer therapy. Anticancer Agents Med. Chem. 11, 38–46.
[34] Lambrecht, C., Haesen, D., Sents, W., Ivanova, E. and Janssens, V. (2013) Structure, regulation, and pharmacological modulation of PP2A phosphatases. Methods Mol. Biol. 1053, 283–305.