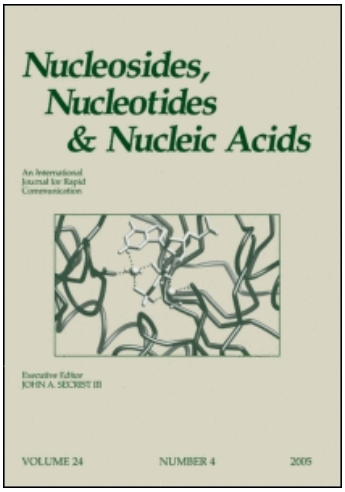

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Thymidine Kinase 1 Deficient Cells Show Increased Survival Rate After UV-Induced DNA Damage

T. Skovgaard[a]; L. J. Rasmussen[a]; B. Munch-Petersen[a]
[a] Department of Science Systems and Models, Roskilde University, Roskilde, Denmark

Online publication date: 11 June 2010

To cite this Article Skovgaard, T. , Rasmussen, L. J. and Munch-Petersen, B.(2010) 'Thymidine Kinase 1 Deficient Cells Show Increased Survival Rate After UV-Induced DNA Damage', Nucleosides, Nucleotides and Nucleic Acids, 29: 4, 347 — 351
To link to this Article: DOI: 10.1080/15257771003741091
URL: http://dx.doi.org/10.1080/15257771003741091

Nucleosides, Nucleotides and Nucleic Acids, 29:347–351, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003741091

Taylor & Francis
Taylor & Francis Group

THYMIDINE KINASE 1 DEFICIENT CELLS SHOW INCREASED SURVIVAL RATE AFTER UV-INDUCED DNA DAMAGE

T. Skovgaard, L. J. Rasmussen, and B. Munch-Petersen
Department of Science Systems and Models, Roskilde University, Roskilde, Denmark

□ *Balanced deoxynucleotide pools are known to be important for correct DNA repair, and deficiency for some of the central enzymes in deoxynucleotide metabolism can cause imbalanced pools, which in turn can lead to mutagenesis and cell death. Here we show that cells deficient for the thymidine salvage enzyme thymidine kinase 1 (TK1) are more resistant to UV-induced DNA damage than TK1 positive cells although they have thymidine triphosphate (dTTP) levels of only half the size of control cells. Our results suggest that higher thymidine levels in the TK- cells caused by defect thymidine salvage to dTTP protects against UV irradiation.*

Keywords Thymidine kinase 1; survival; DNA damage; UV; dTTP pools

INTRODUCTION

When mammalian cells are subjected to DNA damage by ultraviolet (UV)-radiation they respond by cell cycle arrest and initiation of DNA repair, and if the damage is too extensive they enter apoptosis.[1] Balanced deoxynucleotide (dNTP) pools are important for correct DNA repair and replication, and pool imbalances can lead to severe mutagenesis and even cell death.[2]

The most common form of UV-induced DNA damage is the formation of pyrimidine dimers of which T-T dimers formed between two thymines are the most abundant. It has been shown that elevation of thymidine (dThd) levels in the micromolar range stimulated the rejoining of UV-induced DNA-strand breaks in quiescent lymphocytes.[3] dThd is phosphorylated by thymidine kinase 1 (TK1) in cycling cells and by TK2 in resting cells, and two subsequent phosphorylations lead to thymidine triphosphate (dTTP).[4] dTTP

We would like to thank Chiara Rampazzo for providing the HOS cells.

Present address for T. S.: NNF Center for Protein Research, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark.

Present address for L. J. R.: Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark.

Address correspondence to B. Munch-Petersen, Department of Science, Systems and Models, Roskilde University, 4000, Roskilde Denmark. E-mail: bmp@ruc.dk

is a regulator of ribonucleotide reductase (RNR) switching the specificity from pyrimidine to purine nucleotide reduction.[5] It was suggested that the increased DNA repair observed in lymphocytes is caused by a conversion of dThd to dTTP and a subsequent dTTP induced increase of purine dNTPs.[3]

In this study, we examine the connection between cellular dTTP pools and cell survival upon UV irradiation in cycling cells. We compare survival and dTTP pools upon UV irradiation in two osteosarcoma cell lines, one of which has disturbed dTTP metabolism due to TK1 deficiency. An increased survival rate of TK1 deficient (TK^-) cells compared to TK1 proficient (TK^+) control cells is observed, and we suggest that the increased survival is due to increased levels of dThd absorbing some of the UV-radiation in TK^- cells.

MATERIALS AND METHODS

Cell Cultures

HOS and 143B are human osteosarcoma cells originating from the same host. 143B is TK^- and originates indirectly from HOS, which is TK^+. 143B cells (ATCC no.: CRL-8303) were grown in DMEM with Glutamax (GIBCO, Glasgow, Scotland) supplemented with 10% FBS (Biochrom AG, Berlin, Germany), 1% penicillin/streptomycin (GIBCO, Grand Island, NY, USA). HOS cells (ATCC no.: CRL-1543) were grown as 143B cells but with 7.5% FBS.

UV Irradiation of Cells

Prior to UV irradiation, the cells were washed twice with 37°C 1 × Dulbecco's Phosphate Buffered Saline (1xDPBS) containing magnesium and calcium (GIBCO or Lonza). The DPBS was removed and the dishes irradiated at varying time periods with 254 nm UVC light from a 20 $\mu W/cm^2$ source. Unless the cells were harvested immediately after irradiation they were supplemented with preheated medium and stored in the 37°C incubator until harvest.

Cell Survival Assay with Giemsa Staining

An amount of 1,000 and 5,000 cells are seeded in 8.8 cm^2 petri dishes and irradiated on the following day with varying UV doses. The medium is changed every 2–3 days and after 4–6 days, when nonirradiated control plates contain 170–300 colonies of approximately 50 cells, the cells are dried, fixed, and stained as described in Lützen et al.[6] Colonies containing 10–50 cells are counted.

Measuring dTTP Pools

Cells for cell cycle analysis or dTTP assays were set up in 145 cm^2 dishes, and after UV irradiation dTTP pools were measured with the DNA polymerase assay as described in Desler et al.[7] with the following modifications: four times 10 μl assay solution aliquots were spotted on Whatman DE81 paper discs, and after being washed the filters were eluted with 0.5 ml 0.5 M HCl and 0.2 M KCl. Radioactivity was counted in a Wallac Trilux 1450 Microbeta liquid scintillation counter.

Cell Cycle Analysis

Cell cycle distribution was determined with a Becton Dickinson FACS Calibur fluorescence activated cell sorter as described in Lützen et al.[6] Data were analyzed with Cell QuestPro and ModFit software.

RESULTS AND DISCUSSION

Cell survival at varying doses of UV light was determined for the two osteosarcoma cell lines HOS and 143B (Figure 1). HOS and 143B originate from the same tumour but 143B is deficient for the dTTP salvage enzyme TK1. Interestingly, the TK1 deficient cells were more resistant to cell killing by UV than the TK^+ cells, as evident from LD_{50} values of 8 J/m^2 and 4 J/m^2 for 143B and HOS cells, respectively.

Micromolar concentrations of thymidine have previously been reported to increase the repair efficiency of UVC induced DNA strand breaks in quiescent lymphocytes, and it was suggested that this may be due to allosteric regulation of RNR by dTTP.[3]

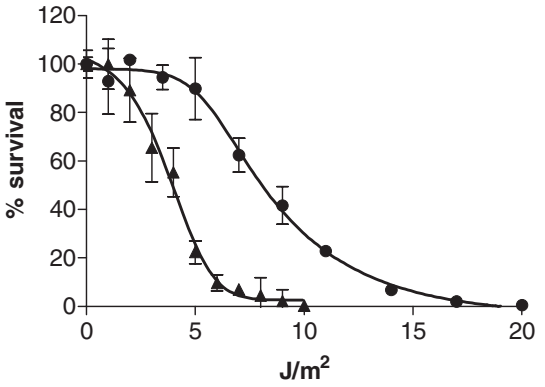

FIGURE 1 Cell survival increase in TK1 deficient osteosarcoma cells. Cell survival at varying doses of UV light. (▲) HOS cells (TK^+) and (●) 143B (TK^-). The LD_{50} values for HOS and 143B cells are 4 J/m^2 and 8 J/m^2, respectively. Error bars are ±SD of three independent measurements.

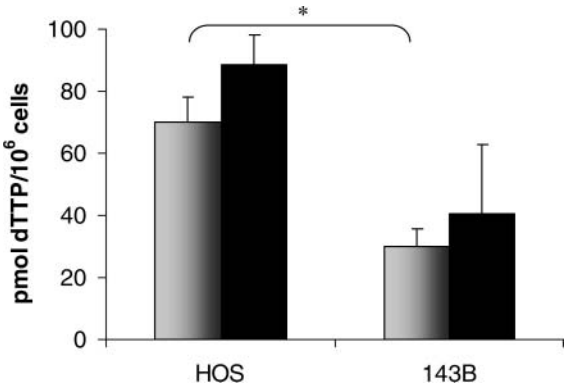

FIGURE 2 dTTP pools are lower in TK^- cells but are not affected by UV irradiation of TK^+ and TK^- osteosarcoma cells. Cellular dTTP levels measured immediately after (grey bars) and 24 hours after (black bars) UV irradiation with a single pulse of 254 nm 20 $\mu W/cm^2$ UV light corresponding to the cells LD50. *dTTP levels at the time of irradiation are significantly different in the two cell lines ($p < 0.05$ with Student's t test). The error bars show ±SD of two independent measurements.

In order to see if the difference in survival rate between the TK^+ and TK^- cells is caused by different dTTP pools the cellular dTTP levels are measured immediately after irradiation and 24 hours after irradiation (Figure 2). The cells are irradiated with a UV dose corresponding to their LD_{50}. At the time of irradiation the TK1 deficient cells have a dTTP pool of half the size of the TK^+ cells. This indicates that TK1 is important for maintaining cellular dTTP pools in cycling cells. The two important regulators of dTTP pools TK1 and RNR are both tightly cell cycle regulated and are only expressed in S-phase. In order to ensure that the differences in dTTP levels are not due to a different number of cells in S-phase and hence different levels of RNR expression, the cell cycle distribution was determined along with the dTTP levels. At the time of UV irradiation the percentage of cells in S-phase was 45 ± 2 and 43 ± 2 for 143B and HOS cells, respectively ($n = 2$, ± *SD*), concluding that the difference in dTTP levels is not due to different percentages of S-phase cells.

The dTTP pools did not change in either of the cell lines when measured 24 hours after UV irradiation. This is in agreement with results previously obtained for TK^+ mouse fibroblasts.[8] Since TK^+ and TK^- cells behave similarly in the period after UV irradiation it is evident that TK1 deficiency does not influence the dTTP pool after DNA damage with UV.

We conclude that the increased survival rate of TK1 deficient cells is not caused by increased dTTP levels, but it is likely that the lack of TK1 in these cells leads to increased levels of dThd since it cannot be phosphorylated. Increased dThd levels have previously been observed in TK1 deficient mice models.[9] It may be that a high level of dThd and not dTTP is the cause of increased repair efficiency in the study on lymphocytes[3] and the increased survival rate in the present study. Since the thymine base of free dThd is able

to absorb part of the UV radiation inflicted on the cells, another possibility is that a high level of dThd in TK^- cells compared to TK^+ cells increases their UV resistance due to higher absorption of UV rays by free dThd.

REFERENCES

1. Latonen, L.; Laiho, M. Cellular UV damage responses functions of tumor suppressor p53. *Biochim. Biophys. Acta* **2005**, 1755(2), 71–89.
2. Kunz, B.A.; Kohalmi, S.E. Modulation of mutagenesis by deoxyribonucleotide levels. *Annu. Rev. Genet.* **1991**, 25, 339–359.
3. Munch-Petersen, B. Thymidine in the micromolar range promotes rejoining of UVC-induced DNA strand breaks and prevents azidothymidine from inhibiting the rejoining in quiescent human lymphocytes. *Mutat. Res.* **1997**, 383(2), 143–153.
4. Arnér, E.S.; Eriksson, S. Mammalian deoxyribonucleoside kinases. *Pharmacol. Ther.* **1995**, 67(2), 155–186.
5. Eriksson, S.; Thelander, L.; Akerman, M. Allosteric regulation of calf thymus ribonucleoside diphosphate reductase. *Biochemistry* **1979**, 18(14), 2948–2952.
6. Lützen, A.; Bisgaard, H.C.; Rasmussen, L.J. Cyclin D1 expression and cell cycle response in DNA mismatch repair-deficient cells upon methylation and UV-C damage. *Exp. Cell. Res.* **2004**, 292(1), 123–134.
7. Desler, C.; Munch-Petersen, B.; Stevnsner, T.; Matsui, S.; Kulawiec, M.; Singh, K.K.; Rasmussen, L.J. Mitochondria as determinant of nucleotide pools and chromosomal stability. *Mutat Res.* **2007**, 625(1–2), 112–124.
8. Håkansson, P.; Hofer, A.; Thelander, L. Regulation of mammalian ribonucleotide reduction and dNTP pools after DNA damage and in resting cells. *J. Biol. Chem.* **2006**, 281(12), 7834–7841.
9. Dobrovolsky, V.N.; Bucci, T.; Heflich, R.H.; Desjardins, J.; Richardson, F.C. Mice deficient for cytosolic thymidine kinase gene develop fatal kidney disease. *Molecular Genetics and Metabolism* **2003**, 78, 1–10.