Purinergic Signalling (2016) 12:575–581
DOI 10.1007/s11302-016-9508-5

BRIEF COMMUNICATION

# Extracellular ATP protects endothelial cells against DNA damage

**Joonas Aho**[1] · **Mikko Helenius**[2] · **Sanna Vattulainen-Collanus**[2] · **Tero-Pekka Alastalo**[2] · **Juha Koskenvuo**[1,3]

Received: 13 October 2015 / Accepted: 8 March 2016 / Published online: 31 March 2016


**Abstract** Cell damage can lead to rapid release of ATP to extracellular space resulting in dramatic change in local ATP concentration. Evolutionary, this has been considered as a danger signal leading to adaptive responses in adjacent cells. Our aim was to demonstrate that elevated extracellular ATP or inhibition of ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1/CD39) activity could be used to increase tolerance against DNA-damaging conditions. Human endothelial cells, with increased extracellular ATP concentration in cell proximity, were more resistant to irradiation or chemically induced DNA damage evaluated with the DNA damage markers γH2AX and phosphorylated p53. In our rat models of DNA damage, inhibiting CD39-driven ATP hydrolysis with POM-1 protected the heart and lung tissues against chemically induced DNA damage. Interestingly, the phenomenon could not be replicated in cancer cells. Our results show that transient increase in extracellular ATP can promote resistance to DNA damage.

Tero-Pekka Alastalo and Juha Koskenvuo are equal last authorship.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11302-016-9508-5) contains supplementary material, which is available to authorized users.

✉ Tero-Pekka Alastalo
tero-pekka.alastalo@helsinki.fi

[1] Research Center of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland

[2] Children's Hospital Helsinki, Pediatric Cardiology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

[3] Department of Clinical Physiology and Nuclear Medicine, HUS Medical Imaging Center, Helsinki University Central Hospital and University of Helsinki, Helsinki, Finland

**Keywords** DNA damage · ATP · NTPDase1 · CD39 · Cancer · Endothelial cell

## Introduction

ATP is an intracellular energy source and an important extracellular signaling molecule. ATP is readily released from various cell types at certain basal rates after cell activation and after cell damage [1, 2]. Burst of ATP functions as evolutionally conserved danger signal for neighboring cells [3]. It is possible that the acute elevation of extracellular ATP is meant to protect surrounding tissue from further damage. Extracellular ATP can promote cellular survival and stimulate proliferation and migration [4, 5]. Several external factors are able to drive cells to apoptosis by destabilizing the nuclear chromatin. It remains obscure whether extracellular ATP could protect cells, such as endothelial cells (EC), against DNA-damaging conditions. Our aim in this study was to establish whether elevated extracellular ATP concentration, through CD39 inhibition, would influence DNA damage sensitivity in ECs.

Double-strand DNA breaks (DSBs) are the most hazardous form of DNA damage as they cause chromosomal rearrangements. Accumulating DSBs may induce apoptosis or cellular dysfunction. DSBs occur at basal levels due to various environmental factors, and approximately 50 endogenous DSBs occur in every cell during cell cycle [6]. Moreover, several cancer therapies such as chemotherapy and gamma irradiation induce DSBs also in non-malignant cells.

ATP and its metabolites act through several cell surface P1 and P2 receptors. Extracellular ATP is readily hydrolyzed by cell membrane-bound and soluble enzymes [7]. The most prominent ATP-hydrolyzing ectoenzyme in endothelial cell (EC) surface is ectonucleoside triphosphate

diphosphohydrolase 1 (ENTPD1/CD39) [8]. It is also highly expressed by other cell types [7]. The overall concentration of extracellular ATP is regulated through ATP release and its hydrolysis by ectoenzymes such as CD39.

The effect of ATP-mediated signaling on DNA damage prevention and repair remains less studied. In this study, we show a protecting role of CD39 attenuation against DNA damage in ECs in vitro and in rat pulmonary and cardiac tissues in vivo. We suggest that targeting the ATP-mediated pathway could represent an attractive strategy for tissue protection during radiotherapy or chemotherapy.

## Materials and methods

### Cell culture

Human pulmonary microvascular ECs (ScienCell, Carlsbad, CA, USA, cat. 3000) were cultured in EBM-2 supplemented with EGM-2 bullet kit (Lonza Clonetics, Walkerville, MD, USA, cat. CC-3162). Human chronic myelogenous leukemia cells (K562, Sigma-Aldrich, Munich, Germany, cat. 89121407) and human diffuse large B cell lymphoma cells (SUDHL-4, ATCC, Teddington, UK, cat. CRL-2957) were cultured in RPMI-1640 (Sigma, cat. R0883) supplemented with 10 % FBS, 2 mM L-glutamine, and 1 % penicillin/streptomycin.

DNA damage was induced with γ-irradiation (4–5 Gy), methyl methanesulfonate (MMS, 500 μM, Sigma, cat. 129925), and doxorubicin hydrochloride (DOX, 1 μM, Tocris, Bristol, UK, cat. 2252). Overnight pretreatments with 10 μM ATP-γ-S (Tocris, cat. 4080) or 100 μM sodium polyoxotungstate (POM-1) (Tocris, cat. 2689) were used.

### RNA interference

Small interfering RNA (siRNA) was used to silence CD39 expression (Dharmacon, cat. L-015973-00-0005). Non-target siRNA was used as a control (Dharmacon, cat. D-001810-01-05). The ECs were siRNA treated according to the previously described method [9]. Previously, it has been demonstrated that the CD39-siRNA is effective in these ECs with qRT-PCR and immunofluoresence stainings [10]. Here, the effectiveness of the CD39 siRNA is shown in protein level (Fig. S1).

### Western immunoblotting

Whole cell or tissue lysates were prepared as previously described [9]. Primary antibodies used are the following: anti-phospho-histone H2A.X (Ser139) 1:1500 (Merck Life Science, Millipore, Espoo, Finland, cat. MABE205), p53 (ser15) 1:1000 (Cell Signaling, Leiden, The Netherlands, cat. 9284), and β-actin 1:2000 (Santa Cruz Biotechnology, Heidelberg, Germany, cat. sc-1615). Secondary antibodies used are the following: goat anti-rabbit IgG-HRP (Santa Cruz Biotechnology, cat. sc-2004) and donkey anti-goat IgG-HRP (Santa Cruz Biotechnology, cat. sc-2020).

### Immunocytochemistry

After 4-h recovery from γ-irradiation, the cells were fixed with 4 % paraformaldehyde and permeabilized with 0.1 % Triton-X-100. Primary antibody was γH2AX (Ser139) 1:100 (Millipore, cat. MABE205). Secondary antibody was Alexa Fluor 488 1:100 (Life technologies, cat. A-21206). The cells were stained with DAPI prior to mounting. The slides were evaluated and photographed under fluorescent microscope, and γH2AX foci in single cells were calculated with ImageJ software (40 cells per condition) as previously described [11].

### Immunohistochemistry

The collected rat heart and lung tissues were fixed with 10 % formalin for 24 h and then transferred to 70 % ethanol. After fixation, the tissues were paraffin embedded and sectioned to microscopy slides. The primary antibody was γH2AX (Ser139) 1:1000 (Millipore), and the secondary antibody was in Rabbit-on-Rodent HRP-Polymer kit (Biocare Medical, Concord, CA, USA, Cat. RMR622), which was used according to the manufacturer's instructions. Tissue samples were analyzed under a microscope, and quantification was done with ImageJ software with IHC Toolbox plugin [12] to determine the ratio between positive and total nuclei.

### Caspase assay

K562 and SUDHL-4 cells were seeded in 96-well plate, $10^4$ cells per well, in supplemented growth media and let to recover overnight. Next, the cells were treated with 100 μM POM-1 or vehicle, let to recover overnight, and then treated with 1 μM DOX or vehicle for 24 h before caspase 3/7 activity measurement (Caspase Glo 3/7 assay, Promega, Nacka, Sweden, cat. G8091).

### Animals

Male Sprague-Dawley (SD) rats (170–200 g, $N = 3$) were used in experiments, which were done with the permission of the National Animal Experiment Board. The rats were given POM-1 (Tocris) (10 mg/kg, intraperitoneal (i.p.)) or PBS in three consecutive days. At the third day, the rats were further treated with monocrotaline (MCT) (60 mg/kg, s.c., Sigma, Cat. 2401), DOX (Tocris) (6 mg/kg, i.p.), or PBS. In MCT group, lungs were collected 24 h after injection, and in the

DOX group, hearts were collected 8 h after injection. In all groups, the rats were euthanized with $CO_2$.

### Statistics

Statistical analysis was done with Prism GraphPad 6 (La Jolla, CA, USA). Unpaired *t* test was used for comparing groups. Results are expressed in mean ± SEM from at least three independent experiments. $P < 0.05$ was considered as significant, and in the figures, *p* values are expressed with stars: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

## Results

### Silencing of CD39 protects ECs from DNA damage

Suppression of CD39 expression in ECs significantly decreased γH2AX expression in both control and γ-irradiated cells when compared to non-target (NT) siRNA-treated cells (Fig. 1a). Compared to control, the γH2AX protein expression was 56 % lower after 45-min recovery ($p = 0.0090$) and 69 % lower after 4-h recovery ($p = 0.0009$) in CD39-deficient cells (Fig. 1a). Supporting results were obtained from immunocytochemistry experiments where the number of γH2AX foci in individual cells (Fig. 1b) was quantified in γ-irradiated cells ($p = 0.0008$). Similarly to irradiation, the expression level of γH2AX in CD39-siRNA silenced cells was 60 % lower after 4-h MMS treatment ($p = 0.0143$), compared to control siRNA-treated cells (Fig. 1c). Results from CD39-deficient cells treated with DOX for 4 h supported the hypothesis ($p = 0.0524$) (Fig. 1c). Similarly, the CD39-deficient ECs treated with MMS had significantly decreased (59 %) expression of phosphorylated p53 protein, a marker of activated DNA damage pathway, when compared to control ($p = 0.0023$, Fig. 1d), while the total p53 protein expression remained unaltered (Fig. S3).

### Both ATP and POM-1 protect ECs from DNA damage

ATP analogue, ATP-γ-S, pretreatment markedly decreased γH2AX expression after irradiation ($p = 0.0592$)- or MMS ($p = 0.0461$)-induced DNA damage (Fig. 2a) compared to control cells. Similarly, CD39 inhibitor POM-1 pretreatment significantly decreased γH2AX expression at basal level ($p = 0.0136$) and after MMS ($p = 0.0036$)-induced DNA damage (up to 80 %, Fig. 2b) compared to control cells. The expression level of serine 15-phosphorylated p53 was suppressed 47 % in POM-1-treated ECs compared to control after exposure to MMS-induced DNA damage ($p = 0.0163$, Fig. 2c), while the total p53 protein expression remained unaltered (Fig. S4).

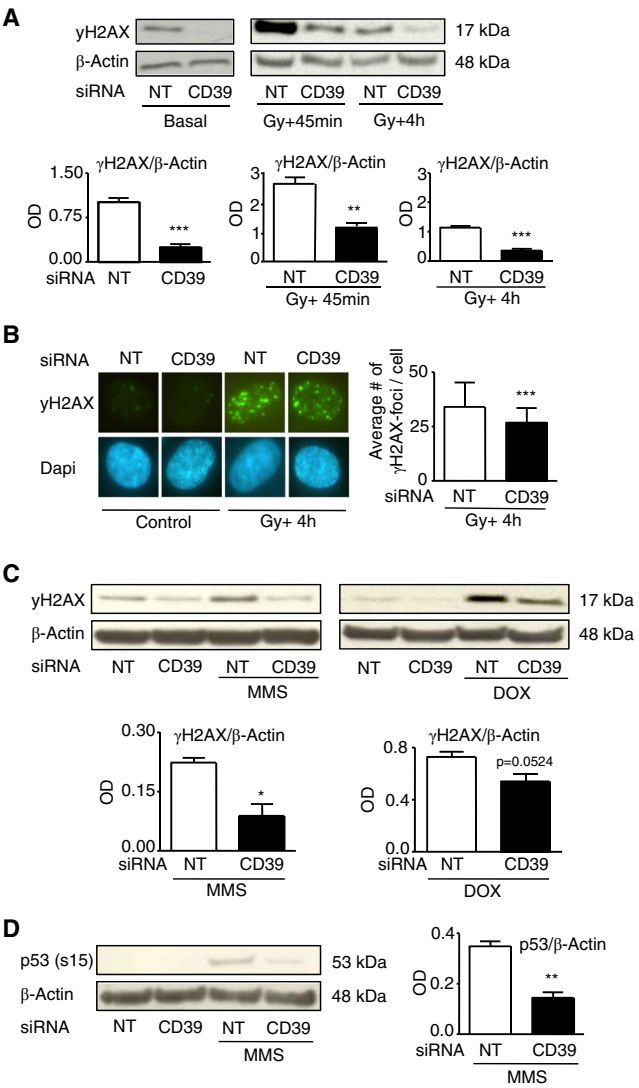


**Fig. 1** CD39 siRNA silenced ECs have decreased γH2AX protein expression after irradiation or chemically induced DNA damage. **a** γH2AX protein expression from CD39 siRNA silenced (CD39) and non-target siRNA (NT)-treated ECs at basal level and after irradiation followed by 45-min or 4-h recovery time. The *bars* represent the optical density (OD) of the γH2AX/β-actin ratio from three independent western immunoblot (WB) experiments. **b** Immunocytochemistry from γH2AX protein expression after irradiation from CD39 siRNA silenced (CD39) and non-target siRNA-treated (NT) ECs. Histogram represents a quantification of average number of γH2AX foci/cell; 40 cells per condition were analyzed with ImageJ. **c** γH2AX protein expression and quantification after MMS- or DOX-induced DNA damage from CD39 siRNA silenced (CD39) and non-target siRNA (NT)-treated ECs. **d** Serine 15 phosphorylated p53 protein expression and quantification after MMS-induced DNA damage from CD39 siRNA silenced (CD39) and non-target siRNA (NT)-treated ECs. β-Actin was used as a loading control in all experiments

### ATPase activity is decreased in CD39 siRNA and POM-1-treated cells

Specific ATPase activity was significantly decreased in POM-1-treated and CD39-deficient ECs compared to control cells

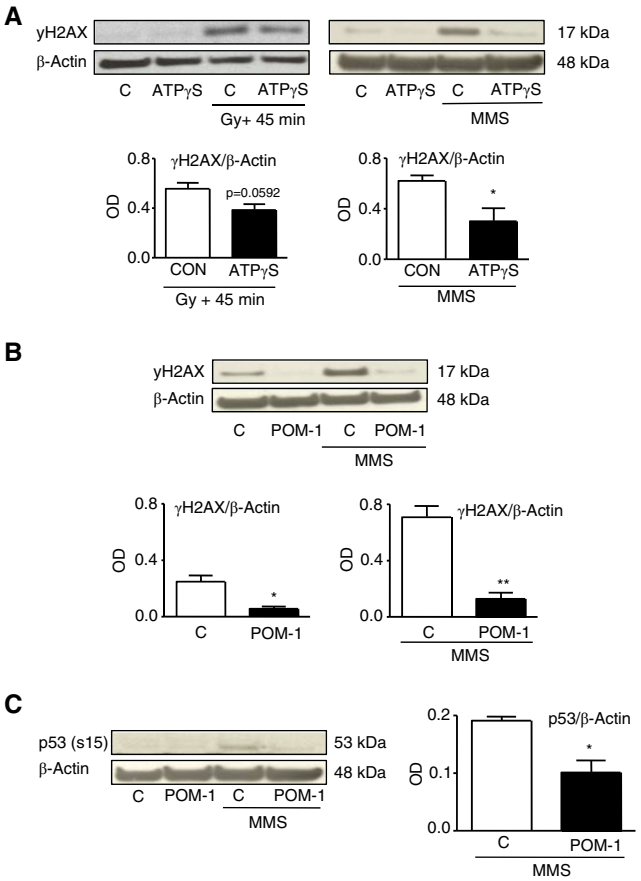


**Fig. 2** ATP stimulation and suppression of CD39 activity decrease γH2AX protein expression after irradiation or chemically induced DNA damage. **a** γH2AX protein expression at basal rate and after irradiation or MMS-induced DNA damage with or without ATPγS pretreatment. Histograms represent quantifications from three independent WB experiments. The *bars* indicate the optical density (OD) of the γH2AX/β-actin ratio. **b** γH2AX protein expression at basal rate and after MMS-induced DNA damage with or without POM-1 treatment. Histograms represent quantifications from three independent WB experiments. The *bars* indicate the optical density (OD) of the γH2AX/β-actin ratio. **c** Serine 15 phosphorylated p53 protein expression at basal rate and after MMS-induced DNA damage with or without POM-1 treatment. Histogram represents quantifications from three independent WB experiments. The *bars* indicate the optical density (OD) of the p53/β-actin ratio. β-Actin was used as a loading control in all experiments

(Fig. S2). Moreover, POM-1 treatment had no additional inhibitory effects on CD39 siRNA silenced cell ATPase activity.

### POM-1 treatment enhanced DNA damage resistance in vivo

To test whether the observed effects of CD39 inhibition and ATP accumulation would apply also in vivo, we used SD rats. The γH2AX expression in lung lysates was significantly lower in MCT animals pretreated with POM-1 compared to control animals (48 %, $p = 0.0109$, Fig. 3a). Similarly, we discovered significantly lower γH2AX expression in heart lysates in DOX-treated rats that were pretreated with POM-1 (53 %, $p = 0.0371$, Fig. 3b). Semiquantitative immunohistochemistry analysis confirmed the result in cardiac cells. The γH2AX expression in cardiac tissue was significantly lower in POM-1-pretreated rats compared to animals, which received DOX without POM-1 pretreatment ($p = 0.0013$, Fig. 3c).

### POM-1 treatment does not rescue cancer cells against DOX treatment

Human leukemia- and lymphoma-derived K562 and SUDHL-4 cells, respectively, were treated with POM-1 and subsequently exposed to DOX for 24 h. Immunoblot analysis of γH2AX expression showed no difference between the groups (Fig. 3d). To test whether CD39 inhibition could protect these cancer cells against DOX-induced apoptosis, we evaluated caspase 3/7 activity after 24-h DOX treatment with or without POM-1 pretreatment. We did not observe any attenuation of caspase 3/7 activity in POM-1 pretreated cells (Fig. 3e). The POM-1 pretreatment significantly increased caspase 3/7 activity after DOX treatment in K562 cells and had no effect in SUDHL-4 cells.

## Discussion

In this study, we show for the first time how suppression of CD39 and resulting elevated extracellular ATP niche [10] can promote resistance to DNA damage under various DNA-damaging conditions in vitro and in vivo. In addition, we demonstrate that inhibition of CD39 does not promote DNA damage repair or apoptosis resistance in transformed cancer cells.

Homologous recombination (HR) and non-homologous end joining (NHEJ) are the two main DNA repair mechanisms used in cells to repair DSBs. The cell cycle phase is the major determinant of the used mechanism. The more effective and less error-prone HR is active only in G2/S phase when the homologous DNA strand can be used as a template [13] while NHEJ operates in all cell cycle phases [14]. In this study, DNA damage was induced with four different DNA DSB-producing mechanisms. The high-energy γ-irradiation breaks the DNA strands directly and mainly involves the NHEJ repair pathway [15, 16]. It is not fully confirmed whether MMS directly induces DSBs, but it stalls DNA replication and HR is involved in the repair of stalled replication forks [17]. DOX induces DNA damage through increased oxidative stress and by intercalating the DNA strands [18]. MCT is metabolized to genotoxic MCT pyrrole and to (+/−)6,7-dihydro-7-hydroxy-1-hydroxymethyl-5H-pyrrolizine (DHP) in vivo, which leads to DNA crosslink and DHP-DNA adduct formation, which are repaired by Fanconi anemia pathway following HR [19, 20].

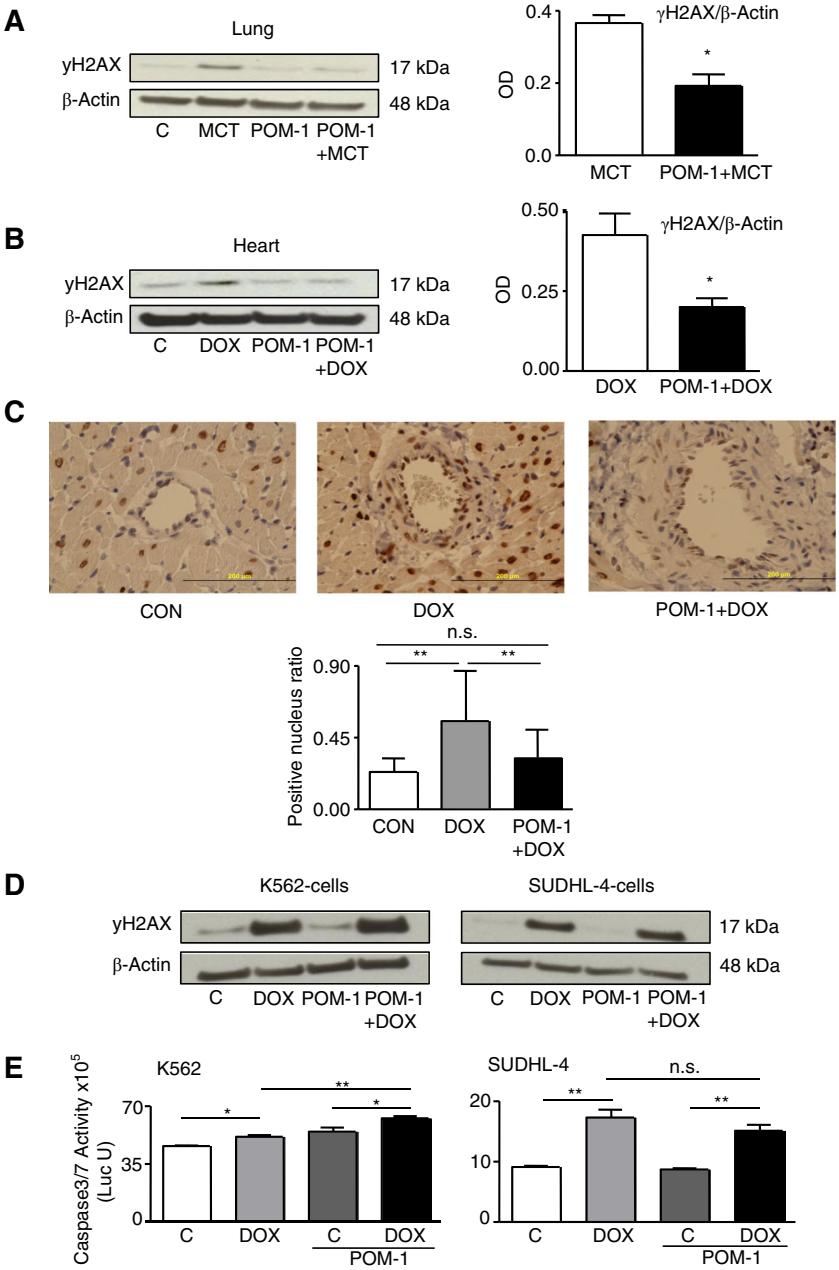


**Fig. 3** CD39 activity inhibition decreases γH2AX protein expression in vivo after chemically induced DNA damage. **a** γH2AX protein expression from lung tissue lysates with control (C) and after MCT-induced DNA damage with or without POM-1 treatment. Histogram represents quantifications from three independent WB experiments. The *bars* indicate the optical density (OD) of the γH2AX/β-actin ratio. **b** γH2AX protein expression from heart tissue lysates with control (C) and after DOX-induced DNA damage with or without POM-1 treatment. Histogram represents quantifications from three independent WB experiments. The *bars* indicate the optical density (OD) of the γH2AX/β-actin ratio. **c** Heart tissue γH2AX protein expression in IHC with control (C) and after DOX-induced DNA damage with or without POM-1 treatment. The foci and the density of foci represent the amount of DSB in single cell, and the histogram represents the ratio of γH2AX nuclei to all nuclei. *Scale bar* 200 μm. **d** γH2AX protein expression from two cancer cell line (K562 and SUDHL-4) lysates with control (C) and after DOX-induced DNA damage with or without POM-1 treatment. β-Actin was used as a loading control in all WB experiments. **e** Caspase 3/7 activity in two cancer cell lines pretreated with 100 μM POM-1 prior to 1-μM DOX treatment for 24 h

Cells are constantly exposed to DSBs that need to be effectively repaired to ensure the normal cell function [6]. DNA damages stall the DNA replication and cell cycle and inhibit cell proliferation. Moreover, accumulated DNA damages can drive cells to apoptosis. As extracellular ATP has been shown to induce DNA replication and cell proliferation in ECs and in smooth muscle cells [4, 21], it could be plausible that these effects are partly mediated through enhanced DNA damage repair. ATP-induced cell proliferation might have a connection to enhanced DNA damage repair as our results demonstrate that basal levels of γH2AX were lower in ATP-activated cells.

Suppression of CD39 not only increases the extracellular ATP but also leads to decreased extracellular adenosine levels in ECs [4]. Adenosine is widely considered to be anti-inflammatory and protective toward vasculature. On the other hand, sustained high adenosine concentration can also be harmful and pro-apoptotic to lung ECs [22]. In CD39-siRNA and POM-1-treated ECs, the decreased adenosine could contribute to DNA damage sensitivity. However, ATP-γ-S treatment, considered not affecting significantly adenosine levels, showed results consistent with CD39 suppressed cells. Other growth factor signaling pathways have been shown to enhance DSB repair after γ-irradiation. Epidermal growth factor receptor variant III (EGFRvIII) signaling has been shown to have a key role in the radioresistance in glioblastoma. EGFRvIII is known to activate downstream

effectors such as phosphatidylinositol 3-kinase (PI3K), Akt-1, Ras, and mitogen-activated protein kinase (MAPK) [23–25]. This downstream signaling eventually leads to DNA-dependent protein kinase (DNA-PKcs) hyperactivation and enhanced DSB repair [25]. Interestingly, ATP has been shown to activate the same downstream signaling pathways and even function in synergy with EGF receptor signaling [26–28].

The few previous studies connecting purinergic signaling to DNA damage repair have been mainly done with cancer cell lines, such as lung cancer. Previous study demonstrated that ATP sensitizes cancer cells to irradiation-induced DNA damage through P2Y6 and P2Y12 receptor activation [29, 30]. In a wider study with six different cancer cell lines, ATP treatment protected against DOX-induced cytotoxicity only in non-metastatic CL1.0 lung cancer cells [31]. Few studies have shown that extracellular ATP promotes survival in non-small cell lung cancer A549 cell line [32, 33]. However, this has not been shown to be a cause of enhanced DNA damage resistance. The distinct purine receptor representation in cancer- and non-malignant cells could explain the difference in cellular response [34]. Other explanation could be difference in purine-inactivating cell surface enzymes between ECs and cancer cells. While CD39 is the main ATP-hydrolyzing enzyme in ECs, cancer cells have additional phosphatases, which could explain the differential response to CD39 inhibition with POM-1 [35]. Our results with cancer cells, where γH2AX expression in POM-1-pretreated cells was increased after DOX-induced DNA damage, fit well to these previous findings. In addition, CD39 inhibition with POM-1 was not able to rescue the cancer cells from DOX-induced cytotoxicity (Fig. 3e). As opposite to cancer cells, in circulating blood cells, others have described that ATP inhibits the radiation-induced DNA damage ex vivo [36].

The great improvements in early cancer detection and cancer treatment strategies have decreased the rate of cancer-related deaths over the last decades [37]. Unfortunately, at the same time, the risk of late-onset cardiovascular complications is increased due to chemotherapy and radiotherapy [38]. Currently, cardiovascular morbidity is the most common non-malignant cause of death among the cancer survivors [39].

Our study, together with previous observations, indicates that there is a profound difference in ATP signaling between cancer cells and non-cancer cells. Considering the protective actions of transient elevation of extracellular ATP in non-cancer cells, we reason this as an attractive strategy for tissue protection during cancer treatments. Additional research is still needed to discover the full mechanism of ATP signaling-mediated resistance to DNA damage in quiescent differentiated cells. Future studies are also needed to better understand the differences in ATP responses between cancer and non-cancer cells and whether certain cancer cells are responsive to ATP similarly than differentiated cells. We propose that targeting and inhibiting CD39 activity could be an attractive strategy to suppress especially cardiovascular injury associated with cancer treatments. Larger in vivo experimental series are now needed to further evaluate the clinical utility of this observation.

**Acknowledgments** This work was supported by Alfred Kordelin Foundation, Sigrid Juselius Foundation, Pediatric Research Foundation Finland, and The Finnish Foundation for Cardiovascular Research.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.

## References

1. Lohman AW, Billaud M, Isakson BE (2012) Mechanisms of ATP release and signalling in the blood vessel wall. Cardiovasc Res 95: 269–280
2. Burnstock G (2006) Purinergic signalling. Br J Pharmacol 147(Suppl 1):S172–S181
3. Naviaux RK (2014) Metabolic features of the cell danger response. Mitochondrion 16:7–17
4. Yegutkin GG, Helenius M, Kaczmarek E, Burns N, Jalkanen S, Stenmark K, Gerasimovskaya EV (2011) Chronic hypoxia impairs extracellular nucleotide metabolism and barrier function in pulmonary artery vasa vasorum endothelial cells. Angiogenesis 14:503–513
5. Burnstock G (2002) Purinergic signaling and vascular cell proliferation and death. Arterioscler Thromb Vasc Biol 22:364–373
6. Vilenchik MM, Knudson AG (2003) Endogenous DNA double-strand breaks: production, fidelity of repair, and induction of cancer. Proc Natl Acad Sci U S A 100:12871–12876
7. Yegutkin GG (2008) Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. Biochim Biophys Acta 1783:673–694
8. Kaczmarek E, Koziak K, Sevigny J, Siegel JB, Anrather J, Beaudoin AR, Bach FH, Robson SC (1996) Identification and characterization of CD39/vascular ATP diphosphohydrolase. J Biol Chem 271:33116–33122
9. Spiekerkoetter E, Guignabert C, de Jesus Perez V, Alastalo TP, Powers JM, Wang L, Lawrie A, Ambartsumian N, Schmidt AM, Berryman M, Ashley RH, Rabinovitch M (2009) S100A4 and bone morphogenetic protein-2 codependently induce vascular smooth muscle cell migration via phospho-extracellular signal-regulated kinase and chloride intracellular channel 4. Circ Res 105:639–647, 13 p following 647
10. Helenius MH, Vattulainen S, Orcholski M, Aho J, Komulainen A, Taimen P, Wang L, de Jesus Perez VA, Koskenvuo JW, Alastalo TP (2015) Suppression of endothelial CD39/ENTPD1 is associated with pulmonary vascular remodeling in pulmonary arterial hypertension. Am J Physiol Lung Cell Mol Physiol, ajplung.00340.2014
11. Light Microscope Core Facility, Duke University and Duke University Medical Centre. How to count nuclear foci in FIJI ImageJ. http://microscopy.duke.edu/HOWTO/countfoci.html. Date last accessed: 03/02 2016
12. Shu J, Qiu G, Ilyas M. Immunohistochemistry (IHC) image analysis toolbox. http://rsb.info.nih.gov/ij/plugins/ihc-toolbox/index.html. 2014. Date last accessed: 09 2015

13. Kakarougkas A, Jeggo PA (2014) DNA DSB repair pathway choice: an orchestrated handover mechanism. Br J Radiol 87: 20130685
14. Lieber MR (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem 79:181–211
15. Rothkamm K, Kuhne M, Jeggo PA, Lobrich M (2001) Radiation-induced genomic rearrangements formed by nonhomologous end-joining of DNA double-strand breaks. Cancer Res 61:3886–3893
16. Hufnagl A, Herr L, Friedrich T, Durante M, Taucher-Scholz G, Scholz M (2015) The link between cell-cycle dependent radiosensitivity and repair pathways: a model based on the local, sister-chromatid conformation dependent switch between NHEJ and HR. DNA Repair (Amst) 27:28–39
17. Lundin C, North M, Erixon K, Walters K, Jenssen D, Goldman AS, Helleday T (2005) Methyl methanesulfonate (MMS) produces heat-labile DNA damage but no detectable in vivo DNA double-strand breaks. Nucleic Acids Res 33:3799–3811
18. Kurz EU, Douglas P, Lees-Miller SP (2004) Doxorubicin activates ATM-dependent phosphorylation of multiple downstream targets in part through the generation of reactive oxygen species. J Biol Chem 279:53272–53281
19. Wagner JG, Petry TW, Roth RA (1993) Characterization of monocrotaline pyrrole-induced DNA cross-linking in pulmonary artery endothelium. Am J Physiol 264:L517–L522
20. Wang YP, Yan J, Beger RD, Fu PP, Chou MW (2005) Metabolic activation of the tumorigenic pyrrolizidine alkaloid, monocrotaline, leading to DNA adduct formation in vivo. Cancer Lett 226:27–35
21. Erlinge D (1998) Extracellular ATP: a growth factor for vascular smooth muscle cells. Gen Pharmacol 31:1–8
22. Lu Q, Sakhatskyy P, Newton J, Shamirian P, Hsiao V, Curren S, Gabino Miranda GA, Pedroza M, Blackburn MR, Rounds S (2013) Sustained adenosine exposure causes lung endothelial apoptosis: a possible contributor to cigarette smoke-induced endothelial apoptosis and lung injury. Am J Physiol Lung Cell Mol Physiol 304: L361–L370
23. Narita Y, Nagane M, Mishima K, Huang HJ, Furnari FB, Cavenee WK (2002) Mutant epidermal growth factor receptor signaling down-regulates p27 through activation of the phosphatidylinositol 3-kinase/Akt pathway in glioblastomas. Cancer Res 62:6764–6769
24. Montgomery RB, Moscatello DK, Wong AJ, Cooper JA, Stahl WL (1995) Differential modulation of mitogen-activated protein (MAP) kinase/extracellular signal-related kinase kinase and MAP kinase activities by a mutant epidermal growth factor receptor. J Biol Chem 270:30562–30566
25. Mukherjee B, McEllin B, Camacho CV, Tomimatsu N, Sirasanagandala S, Nannepaga S, Hatanpaa KJ, Mickey B, Madden C, Maher E, Boothman DA, Furnari F, Cavenee WK, Bachoo RM, Burma S (2009) EGFRvIII and DNA double-strand break repair: a molecular mechanism for radioresistance in glioblastoma. Cancer Res 69:4252–4259
26. Wagstaff SC, Bowler WB, Gallagher JA, Hipskind RA (2000) Extracellular ATP activates multiple signalling pathways and potentiates growth factor-induced c-fos gene expression in MCF-7 breast cancer cells. Carcinogenesis 21:2175–2181
27. Montiel M, de la Blanca EP, Jimenez E (2006) P2Y receptors activate MAPK/ERK through a pathway involving PI3K/PDK1/PKC-zeta in human vein endothelial cells. Cell Physiol Biochem 18:123–134
28. Gerasimovskaya EV, Woodward HN, Tucker DA, Stenmark KR (2008) Extracellular ATP is a pro-angiogenic factor for pulmonary artery vasa vasorum endothelial cells. Angiogenesis 11:169–182
29. Ide S, Nishimaki N, Tsukimoto M, Kojima S (2014) Purine receptor P2Y6 mediates cellular response to gamma-ray-induced DNA damage. J Toxicol Sci 39:15–23
30. Nishimaki N, Tsukimoto M, Kitami A, Kojima S (2012) Autocrine regulation of gamma-irradiation-induced DNA damage response via extracellular nucleotides-mediated activation of P2Y6 and P2Y12 receptors. DNA Repair (Amst) 11:657–665
31. Wang JS, Chang YL, Yu YH, Chen CY, Kao MC, Li TK, Lin WW (2012) Cell type-specific effects of adenosine 5′-triphosphate and pyrophosphate on the antitumor activity of doxorubicin. Cancer Sci 103:1811–1819
32. Qian Y, Wang X, Liu Y, Li Y, Colvin RA, Tong L, Wu S, Chen X (2014) Extracellular ATP is internalized by macropinocytosis and induces intracellular ATP increase and drug resistance in cancer cells. Cancer Lett 351:242–251
33. Song S, Jacobson KN, McDermott KM, Reddy SP, Cress AE, Tang H, Dudek SM, Black SM, Garcia JG, Makino A, Yuan JX (2016) ATP promotes cell survival via regulation of cytosolic [Ca2+] and Bcl-2/Bax ratio in lung cancer cells. Am J Physiol Cell Physiol 310: C99–C114
34. Puchalowicz K, Tarnowski M, Baranowska-Bosiacka I, Chlubek D, Dziedziejko V (2014) P2X and P2Y receptors-role in the pathophysiology of the nervous system. Int J Mol Sci 15:23672–23704
35. Helenius M, Jalkanen S, Yegutkin GG (2012) Enzyme-coupled assays for simultaneous detection of nanomolar ATP, ADP, AMP, adenosine, inosine and pyrophosphate concentrations in extracellular fluids. Biochim Biophys Acta (BBA) - Mol Cell Res 1823: 1967–1975
36. Swennen EL, Dagnelie PC, Van den Beucken T, Bast A (2008) Radioprotective effects of ATP in human blood ex vivo. Biochem Biophys Res Commun 367:383–387
37. Siegel R, Ma J, Zou Z, Jemal A (2014) Cancer statistics, 2014. CA Cancer J Clin 64:9–29
38. Altena R, Perik PJ, van Veldhuisen DJ, de Vries EG, Gietema JA (2009) Cardiovascular toxicity caused by cancer treatment: strategies for early detection. Lancet Oncol 10:391–399
39. Jaworski C, Mariani JA, Wheeler G, Kaye DM (2013) Cardiac complications of thoracic irradiation. J Am Coll Cardiol 61:2319–2328