Original Paper

A_3 Adenosine Receptor Mediates Apoptosis in 5637 Human Bladder Cancer Cells by G_q Protein/PKC-Dependent AIF Upregulation

Takeshi Kanno[a]   Akinobu Gotoh[b]   Yumiko Fujita[a]   Takashi Nakano[c]
Tomoyuki Nishizaki[a]

[a]Division of Bioinformation, Department of Physiology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya; [b]Laboratory of Cell and Gene Therapy, Institute for Advanced Medical Sciences, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya; [c]Department of Thoracic Oncology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya

Abstract

Background/Aims: A_3 adenosine receptor mediates apoptosis in a variety of cancer cells via diverse signaling pathways. The present study was conducted to assess A_3 adenosine receptor-mediated apoptosis in human bladder cancer cell lines and to understand the underlying mechanism. ***Methods:*** Human bladder cancer cell lines such as 253J, 5637, KK-47, TCCSUP, T24, and UMUC-3 cells were cultured. The siRNA to silence the A_3 adenosine receptor-targeted gene was constructed and transfected into cells. MTT assay, TUNEL staining, Western blotting, and real-time RT-PCR were carried out. ***Results:*** For all the investigated cell types adenosine induced apoptosis in a concentration (0.01-10 mM)- and treatment time (24-48 h)-dependent manner. Adenosine-induced 5637 cell death was significantly inhibited by the A_3 adenosine receptor inhibitor MRS1191 or knocking-down A_3 adenosine receptor, and the A_3 adenosine receptor agonist 2-Cl-IB-MECA mimicked the adenosine effect. The adenosine effect was prevented by GF109203X, an inhibitor of protein kinase C (PKC), but it was not affected by forskolin, an activator of adenylate cyclase. Adenosine-induced 5637 cell death, alternatively, was not inhibited by the pan-caspase inhibitor Z-VAD. Adenosine upregulated expression of apoptosis-inducing factor (AIF), that is suppressed by knocking-down A_3 adenosine receptor, and accumulated AIF in the nucleus. ***Conclusion:*** The results of the present study show that adenosine induces 5637 cell apoptosis by upregulating AIF expression via an A_3 adenosine receptor-mediated G_q protein/PKC pathway.

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Prof. Tomoyuki Nishizaki

Division of Bioinformation, Department of Physiology,
Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya 663-8501 (Japan)
Tel. +81-798-45-6397, Fax +81-798-45-6649, E-Mail tomoyuki@hyo-med.ac.jp

Introduction

Apoptosis is induced in a caspase-dependent and -independent manner. Apoptosis-inducing factor (AIF), that is localized in the mitochondria under the normal conditions, is implicated in caspase-independent apoptosis. Apoptotic signals trigger to release AIF from the mitochondria and deliver into the nucleus, where AIF binds to the nuclear DNA, causing chromosomal condensation, margination, and large-scale DNA fragmentation, to induce apoptosis [1-3]. As is the case with AIF, AIF-homologous mitochondrion-associated inducer of death (AMID), a human pro-apoptotic protein, also induces caspase-independent apoptosis by accumulating in the nucleus from the mitochondria or the cytoplasm [4].

Extracellular adenosine induces apoptosis in a variety of cancer cells through an intrinsic pathway relevant to adenosine uptake into cells and an extrinsic pathway relevant to adenosine receptors. Intracellularly transported adenosine induces apoptosis in MCF-7 human breast cancer cells by accumulating AMID in the nucleus [5]. Adenosine induces apoptosis in HuH-7 human hepatoma cells by upregulating AMID expression [6]. AMP converted from intracellularly transported adenosine induces apoptosis in GT3-TKB human lung cancer cells and HuH-7 cells by activating AMP-activated protein kinase (AMPK) [7, 8] or in malignant pleural mesothelioma cells by upregulating p53 expression [9]. Intracellularly transported adenosine, alternatively, downregulates expression of c-FLIP to neutralize caspase-8 inhibition due to c-FLIP, resulting in the activation of caspase-8 and the effector caspase-3, responsible for HuH-7 cell apoptosis [10]. Intracellularly transported adenosine still activates caspase-3 by neutralizing caspase-3 inhibition due to inhibitor of apoptosis protein (IAP) as a result of decreased IAP2 expression and reduced IAP activity in response to increased DIABLO expression and DIABLO release from damaged mitochondria in HuH-7 cells, regardless of caspase-9 activation [11].

Adenosine receptors include A_1, A_{2a}, A_{2b}, and A_3 receptors. A_1 adenosine receptor is linked to G_i protein involving adenylate cyclase inhibition. A_{2a} and A_{2b} adenosine receptors are linked to G_s protein involving adenylate cyclase activation. A_3 adenosine receptor is linked to G_i or G_q protein involving phospholipase C activation [12]. Adenosine induced apoptosis in CW2 human colonic cancer cells by activating caspase-3, -8, and -9 via A_1 adenosine receptor [13]. A_1 adenosine receptor participated in RCR-1 astrocytoma cell apoptosis [14]. Adenosine induces apoptosis in Caco-2 human colonic cancer cells by activating caspase-9/-3 via A_{2a} adenosine receptor [15]. Adenosine induces apoptosis in A549 human lung cancer cells by upregulating expression of Bax, Bad, and Puma, to disrupt mitochondrial membrane potentials and to activate caspase-9 followed by the effector caspase-3 [16], in Lu-65 human lung cancer cells by upregulating p53 expression [17], and in SBC-3 human lung cancer cells and RCC4-VHL human renal cancer cells by upregulating AMID expression [18, 19], all as mediated via A_3 adenosine receptor.

The present study investigated adenosine-induced apoptosis in human bladder cancer cell lines such as 253J, 5637, KK-47, T24, TCCSUP, and UMUC-3 cells. We show here that adenosine induces apoptosis in all the bladder cancer cell lines via different pathways and that A_3 adenosine receptor mediates 5637 cell apoptosis by G_q protein/PKC-dependent AIF upregulation.

Materials and Methods

Cell Culture

Human bladder cancer cell lines used here were 253J, 5637, KK-47, T24, TCCSUP, and UMUC-3 cells. KK-47 was generously provided by Dr. Seiji Naito (Department of Urology, Kyushu University, Fukuoka, Japan), and TCCSUP, T24, 253J, UMUC-3, and 5637 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells were grown in Roswell Park Memorial Institute-1640 (Life Technologies, Gaithersburg, MD USA) supplemented with 10% (v/v) heat-inactivated fetal bovine serum, penicillin (final concentration, 100 U/ml), and streptomycin (final concentration, 0.1 mg/ml), in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

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1161

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Table 1. Primers used for real-time RT-PCR.

PCR primers	Oligonucleotide sequence
AIF	Sense: 5'-TCACAAAGACACTGCGATTCAAACAGT-3'
	Anti-sense: 5'-GTTGCTGAGGTATTCGGGGAGGAT-3'
AMID	Sense: 5'-GGGTTCGCCAAAAAGACATTCATTT-3'
	Anti-sense: 5'-CCTCTGTGCCTTTGTCCGTCTGC-3'
Bad	Sense: 5'-CTGGGGCTGTGGAGATCCGGAGTCGCC-3'
	Anti-sense: 5'-TCACTGGGAGGGGGCGGAGCTTCCCC-3'
Bax	Sense: 5'-CGGACCCGGCGAGAGGC-3'
	Anti-sense: 5'-TCAGCTTCTTGGTGGACGCATCC-3'
Bid	Sense: 5'-CTACGATGAGCTGCAGACTG-3'
	Anti-sense: 5'-GATGCTACGGTCCATGCTGTC-3'
Puma	Sense: 5'-GACGACCTCAACGCACAGTA-3'
	Anti-sense: 5'-AGGAGTCCCATGATGAGATTGT-3'
Hrk	Sense: 5'-TGCTCGGCAGGCGGAACTTGTAG-3'
	Anti-sense: 5'-CTTTCTCCAAGGACACAGGG-3'
Noxa	Sense: 5'-GCAGAGCTGGAAGTCGAGTG-3'
	Anti-sense: 5'-GAGCAGAAGAGTTTGGATATCAG-3'
Bcl-2	Sense: 5'-TCCGCATCAGGAAGGCTAGA-3'
	Anti-sense: 5'-AGGACCAGGCCTCCAAGCT-3'
Bcl-X_L	Sense: 5'-TGGAATTCATGTCTCAGAGCAACCGGGAGC-3'
	Anti-sense: 5'-CAGAATTCTCATTTCCGACTGAAGAGTGAGC-3'
Mcl-1	Sense: 5'-GGACATCAAAAACGAAGACG-3'
	Anti-sense: 5'-GCAGCTTTCTTGGTTTATGG-3'
p53	Sense: 5'-GCCATCTACAAGCAGTCACAGCACAT-3'
	Anti-sense: 5'-GGCACAAACACGCACCTCAAAGC-3'
GAPDH	Sense: 5'-GACTTCAACAGCGACACCCACTCC-3'
	Anti-sense: 5'-AGGTCCACCACCCTGTTGCTGTAG-3'

Assay of cell viability

Cell viability was assayed by the method using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) as described previously [7].

Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining

TUNEL staining was performed to detect *in situ* DNA fragmentation as a marker of apoptosis using an In Situ Apoptosis Detection Kit (Takara Bio, Otsu, Japan). Briefly, fixed and permeabilized cells were reacted with terminal deoxynucleotidyl transferase and fluorescein isothiocyanate (FITC)-deoxyuridine triphosphate for 90 min at 37 °C. FITC signals were visualized with a confocal scanning laser microscope (LSM 510, Carl Zeiss Co., Ltd., Oberkochen, Germany).

Construction and transfection of siRNA

The siRNA to silence human A_3 adenosine receptor-targeted gene (A_3R siRNA) and the negative control siRNA (NC siRNA) were obtained from Ambion (Austin, TX, USA). The sequences of A_3R siRNA was: 5'-GGGAGUGAAUUGAAUUUAATT-3' and 5'-UUAAAUUCAAUUCACUCCCTG-3'.

The NC siRNA or the A_3R siRNA was reverse-transfected into cells using a Lipofectamine reagent (Invitrogen, Carlsbad, CA, USA). Cells were used for experiments 48 h after transfection.

Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Real-time RT-PCR was carried out using primers shown in Table 1 by the method as described previously [16]. Signal intensities for each mRNA was normalized by those for the GAPDH mRNA.

Separation into the nuclear and cytosolic components

Cells were separated into the nuclear and cytosolic components by the method as previously described [5]. Briefly, lysed cells were centrifuged at 3,500 rpm for 5 min at 4 °C. The pellet and supernatant were used as nuclei- and cytosol-enriched components. Whether the nuclear and cytosolic components were successfully separated was confirmed by Western blotting using an anti-Lamin A/C antibody, a nuclear marker.

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1162

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Western blotting

Western blotting was carried out using an anti-A_1 receptor antibody (Oncogene, Cambridge, MA, USA), an anti-A_{2a} receptor antibody (Oncogene), an anti-A_{2b} receptor antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), an anti-A_3 receptor antibody (Santa Cruz Biotechnology), an anti-AIF antibody (Santa Cruz Biotechnology), or an anti-β-actin antibody (Sigma, St Louis, MO, USA) by the method as previously described [5].

Statistical analysis

Statistical analysis was carried out using unpaired *t*-test and Dunnett's test.

Results

Adenosine induces apoptosis in human bladder cancer cells

For all the bladder cancer cell lines examined here, adenosine reduced cell viability in a concentration (0.01-10 mM)- and treatment time (24-48 h)-dependent manner, to an extent slightly varying among the cell types (Fig. 1A-F). In the TUNEL staining analysis, adenosine significantly increased TUNEL-positive cells as compared with untreated controls for all the cell types (Fig. 2A-F), indicating that adenosine induces apoptosis in bladder cancer cells.

A_3 adenosine receptor mediates 5637 cell apoptosis

Adenosine-induced reduction in cell viability for 253J, KK-47, and T24 cells was little inhibited by 8-cyclopentyltheophylline (8-CPT), an antagonist of A_1 adenosine receptor, 3,7-dimethyl-1-propargylxanthine (DMPX), an antagonist of A_{2a} adenosine receptor, MRS1706, an antagonist of A_{2b} adenosine receptor, MRS1191, an antagonist of A_3 adenosine receptor, or dipyridamole, an inhibitor of adenosine transporter (Fig. 3A, C, D). This suggests less implication of adenosine receptors and adenosine transporter in adenosine-induced apoptosis in 253J, KK-47, and T24 cells.

The adenosine effect on 5637 cell viability was significantly inhibited by MRS1191, but it was not affected by 8-CPT, DMPX, or dipyridamole (Fig. 3B), suggesting the implication of A_3 adenosine receptor in adenosine-induced 5637 cell apoptosis.

The adenosine effect on TCCSUP cell viability was attenuated by all the adenosine receptor inhibitors, with the relative higher potential for DMPX and MRS1706 (Fig. 3E), suggesting the implication of A_2 adenosine receptor in adenosine-induced TCCSUP cell apoptosis.

The adenosine effect on UMUC-3 cell viability was inhibited by all the adenosine receptor inhibitors and dipyridamole, with the highest potential for MRS1706 (Fig. 3F), suggesting the implication of A_{2b} adenosine receptor in adenosine-induced UMUC-3 cell apoptosis.

To understand the mechanism for A_3 adenosine receptor-mediated apoptosis, we focused upon 5637 cells. Like adenosine, 2-chloro-N^6-(3-iodobenzyl)-adenosine-5'-N-methyl-uronamide (2-Cl-IB-MECA), an agonist of the A_3 adenosine receptor, reduced 5637 cell viability (Fig. 4A), supporting the note for the implication of A_3 adenosine receptor in adenosine-induced 5637 cell apoptosis.

To obtain further evidence for the implication of A_3 adenosine receptor, the A_3R siRNA was constructed. 5637 cells express all the adenosine receptor proteins, and expression of the A_3 adenosine receptor protein was evidently suppressed in cells transfected with the A_3R siRNA (Fig. 5A), confirming A_3 adenosine receptor knock-down. Adenosine-induced reduction in 5637 cell viability was significantly prevented for cells transfected with the A_3R siRNA as compared with that for cells transfected with the NC siRNA (Fig. 5B). Moreover, TUNEL-positive cells after adenosine treatment were significantly decreased by knocking-down A_3 adenosine receptor (Fig. 5C). Overall, these results provide evidence for A_3 adenosine receptor-dependent 5637 cell apoptosis.

A_3 adenosine receptor is linked to G_i protein involving adenylate cyclase inhibition and G_q protein involving phospholipase C activation [12]. Adenosine-induced reduction in

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1163

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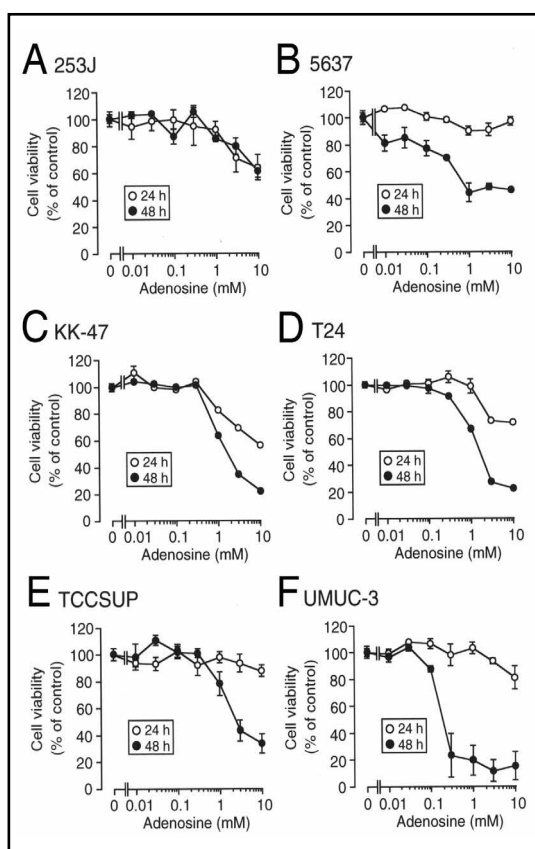
Fig. 1. Adenosine-induced bladder cancer cell death. MTT assay was carried out in 253J (A), 5637 (B), KK-47 (C), T24 (D), TCCSUP (E), and UMUC-3 cells (F) untreated and treated with adenosine at concentrations as indicated for 24-48 h. In the graphs, each point represents the mean (± SEM) percentage of control (MTT intensities of cells untreated with adenosine) (n=4 independent experiments).
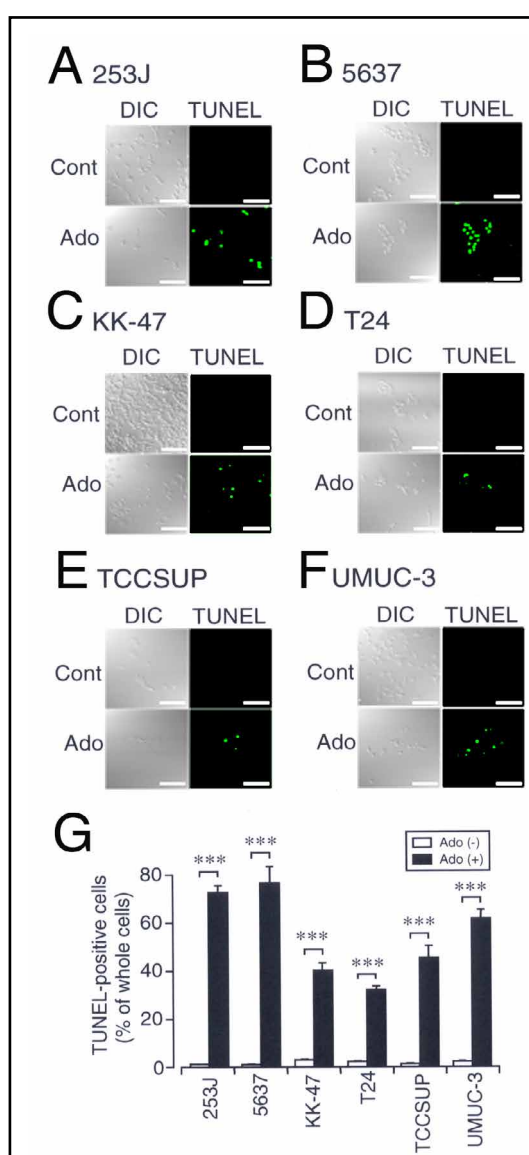

Fig. 2. Adenosine-induced bladder cancer cell apoptosis. TUNEL staining was carried out in 253J (A), 5637 (B), KK-47 (C), T24 (D), TCCSUP (E), and UMUC-3 cells (F) untreated (Cont) and treated with adenosine (Ado)(3 mM) for 48 h. DIC, differential interference contrast. Bars, 100 μm. TUNEL-positive cells were counted in the area (0.4 mm x 0.4 mm) selected at random. (G) In the graphs, each column represents the mean (± SEM) TUNEL-positive cell percentage of whole cells (n=4 independent experiments). ***$P <0.001$, unpaired t-test.

5637 cell viability was significantly inhibited by GF109203X, an inhibitor of PKC, but it was not affected by forskolin, an activator of adenylate cyclase (Fig. 4B). This suggests that A_3 adenosine receptor mediates 5637 cell death dominantly via a G_q protein/PKC signaling pathway.

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1164

Kanno/Gotoh/Fujita/Nakano/Nishizaki: A3 Adenosine Receptor-Mediated Bladder
Cancer Cell Apoptosis

Fig. 3. The effects of inhibitors for adenosine receptors and transporter on adenosine-induced bladder cancer cell death. 253J (A), 5637 (B), KK-47 (C), T24 (D), TCCSUP (E), and UMUC-3 cells (F) were treated with adenosine (3 mM) in the absence and presence of 8-CPT (10 μM), DMPX (10 μM), MRS1706 (50 nM), MRS1191 (10 μM), or dipyridamole (+ Dypri)(10 μM) for 48 h, and then, MTT assay was carried out. In the graphs, each column represents the mean (± SEM) percentage of basal levels (MTT intensities of cells untreated with adenosine in the absence of inhibitors) (n=4 independent experiments). *P* value, Dunnett's test.
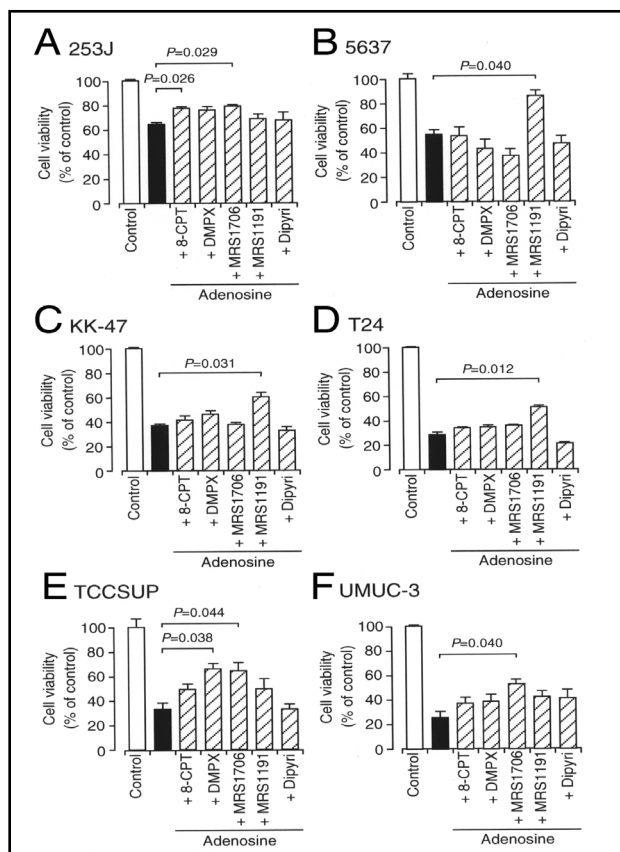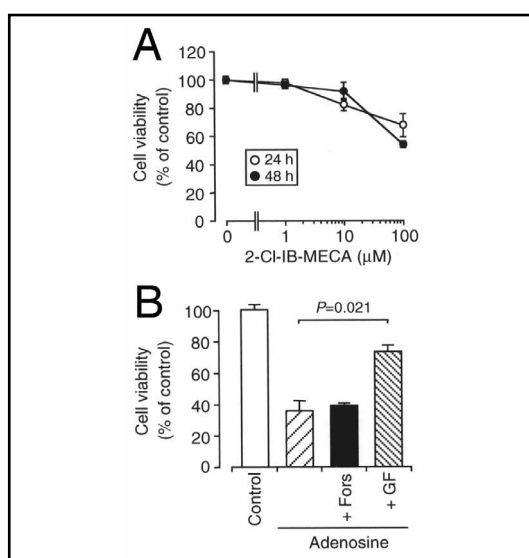

Fig. 4. Adenosine-induced 5637 cell death via A_3 adenosine receptor linked to G_q/PKC pathway. (A) 5637 cells were treated with 2-Cl-IB-MECA at concentrations as indicated for 24-48 h (n=4). In the graph, each point represents the mean (± SEM) percentage of control (MTT intensities of cells untreated with 2-Cl-IB-MECA)(n=4 independent experiments). (B) 5637 cells were treated with adenosine (3 mM) in the presence and absence of forskolin (+ Fors)(10 μM) or GF109203X (+ GF) (100 nM), and then, MTT assay was carried out. In the graph, each column represents the mean (± SEM) percentage of basal levels (MTT intensities of cells untreated with adenosine in the absence of inhibitors)(n=4 independent experiments). *P* value, Dunnett's test.

Adenosine upregulates AIF expression in an A_3 adenosine receptor-dependent manner

Adenosine-induced reduction in 5637 cell viability was not affected by Z-VAD, a pan-caspase inhibitor (Fig. 6), suggesting that adenosine induces 5637 cell apoptosis in a caspase-independent manner.

To probe apoptosis-related factors responsible for adenosine-induced 5637 cell apoptosis, real-time RT-PCR was carried out. Adenosine caused a marked increase in the
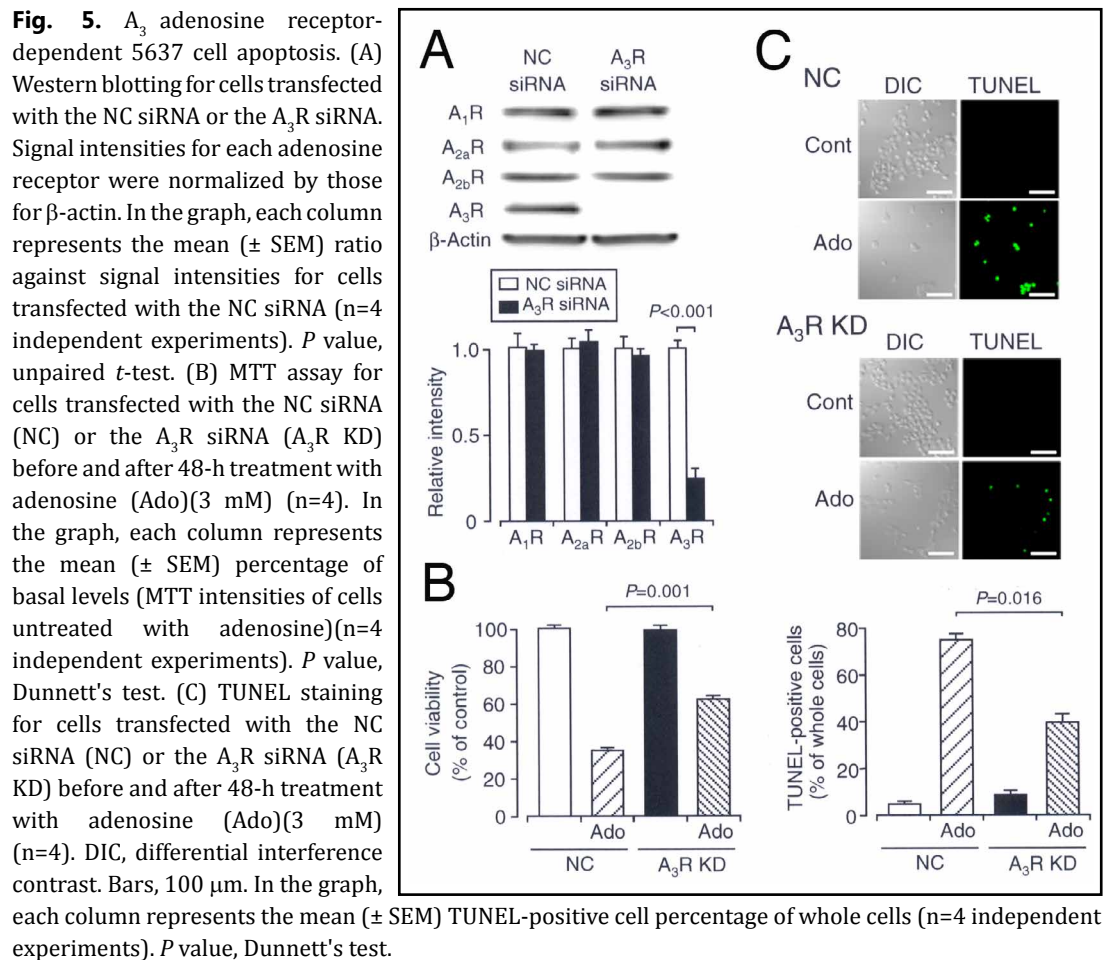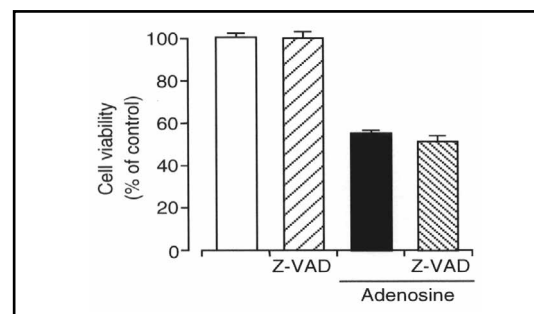
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1165

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Fig. 5. A$_3$ adenosine receptor-dependent 5637 cell apoptosis. (A) Western blotting for cells transfected with the NC siRNA or the A$_3$R siRNA. Signal intensities for each adenosine receptor were normalized by those for β-actin. In the graph, each column represents the mean (± SEM) ratio against signal intensities for cells transfected with the NC siRNA (n=4 independent experiments). *P* value, unpaired *t*-test. (B) MTT assay for cells transfected with the NC siRNA (NC) or the A$_3$R siRNA (A$_3$R KD) before and after 48-h treatment with adenosine (Ado)(3 mM) (n=4). In the graph, each column represents the mean (± SEM) percentage of basal levels (MTT intensities of cells untreated with adenosine)(n=4 independent experiments). *P* value, Dunnett's test. (C) TUNEL staining for cells transfected with the NC siRNA (NC) or the A$_3$R siRNA (A$_3$R KD) before and after 48-h treatment with adenosine (Ado)(3 mM) (n=4). DIC, differential interference contrast. Bars, 100 μm. In the graph, each column represents the mean (± SEM) TUNEL-positive cell percentage of whole cells (n=4 independent experiments). *P* value, Dunnett's test.

Fig. 6. Caspase-independent adenosine-induced apoptosis in 5637 cells. Cells treated with adenosine (3 mM) in the presence and absence of Z-VAD (100 μM) for 48 h, followed by MTT assay. In the graph, each column represents the mean (± SEM) percentage of basal levels (MTT intensities of cells untreated with adenosine in the absence of Z-VAD) (n=4 independent experiments).

expression of the AIF mRNA in 5637 cells, while it did not affect expression of mRNAs for the Bcl-2 family such as Bax, Bad, Bid, Puma, Hrk, Noxa, Bcl-2, Bcl-X$_L$, and Mcl-1, the tumor suppressor p53, and AMID (Fig. 7A). Moreover, adenosine also increased AIF protein in 5637 cells (Fig. 7B). This raises the possibility that AIF is a critical target for adenosine-induced 5637 cell apoptosis.

Adenosine-induced upregulation of expression for the AIF mRNA and protein was significantly prevented by knocking-down A$_3$ adenosine receptor (Fig. 7C, D). This indicates that A$_3$ adenosine receptor promotes the AIF gene transcription in 5637 cells.

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1166

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Fig. 7. Upregulation of AIF expression in an A_3 adenosine receptor-dependent manner. (A) Real-time RT-PCR for apoptosis-related protein mRNAs was carried out in 5637 cells treated with adenosine (3 mM) for periods of treatment time as indicated. Signal intensities for each mRNA were normalized by those for the GAPDH mRNA. In the graphs, each point represents the mean (± SEM) each mRNA intensity (n=4 independent experiments). (B) Western blotting for AIF protein was carried out in 5637 cells treated with adenosine (3 mM) for periods of treatment time as indicated. Signal intensities for AIF protein were normalized by those for β-actin. In the graph, each point represents the mean (± SEM) AIF protein intensity (n=4 independent experiments). Real-time RT-PCR and Western blotting for the AIF mRNA and protein were carried out in 5637 cells transfected with the NC siRNA or the A_3R siRNA, treated with adenosine (3 mM) for 1 and 6 h, respectively. (C) Signal intensities for the AIF mRNA were normalized by those for the GAPDH mRNA. In the graph, each column represents the mean (± SEM) AIF mRNA intensity (n=4 independent experiments). *P* value, Dunnett's test. (D) Signal intensities for AIF protein were normalized by those for β-actin. In the graph, each column represents the mean (± SEM) AIF protein intensity (n=4 independent experiments). *P* value, Dunnett's test.
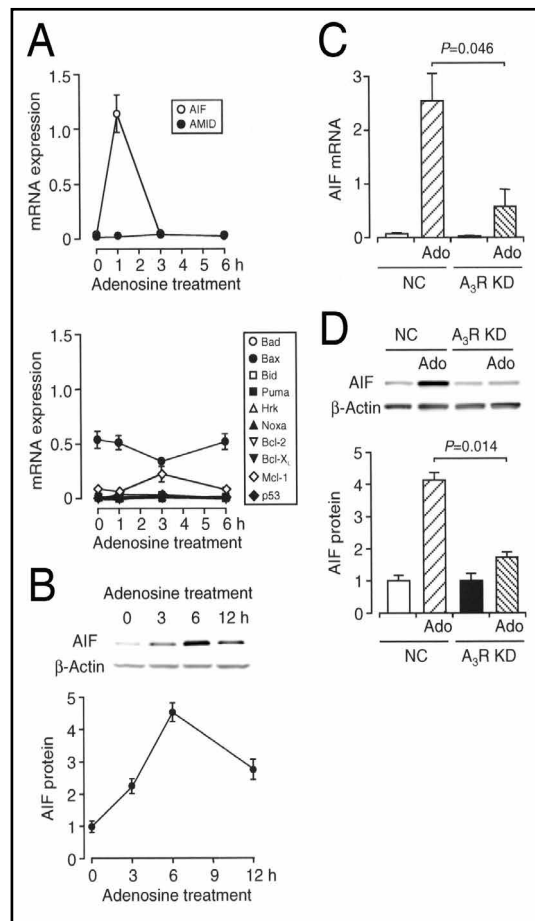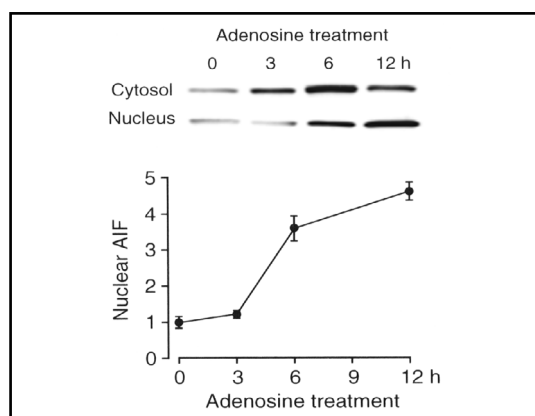

Fig. 8. Intracellular AIF distribution. Western blotting for AIF protein was carried out in 5637 cells treated with adenosine (3 mM) for periods of time as indicated. In the graph, each point represents the mean (± SEM) AIF protein intensity in the nucleus (n=4 independent experiments).

Adenosine accumulates AIF in the nucleus

AIF is recognized to serves as an executioner for caspase-independent apoptosis by accumulating in the nucleus [1-3]. In the analysis of intracellular AIF distribution in 5637 cells, adenosine increased presence of AIF in the nuclear component in parallel with decreased presence in the cytosolic component (Fig. 8). This indicates that adenosine stimulates AIF translocation from the cytosol into the nucleus. Overall, these results lead to a conclusion that adenosine upregulates AIF expression in an A_3 adenosine receptor-dependent manner, possibly linked to G_q protein/PKC activation, and accumulates AIF in the nucleus, to induce 5637 cell apoptosis.

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1167

Kanno/Gotoh/Fujita/Nakano/Nishizaki: A3 Adenosine Receptor-Mediated Bladder Cancer Cell Apoptosis

Discussion

The results of the present study demonstrate that extracellular adenosine induces apoptosis in human bladder cancer cell lines such as 253J, 5637, KK-47, T24, TCCSUP, and UMUC-3 cells, to an different extent, via diverse signaling pathways. Of cell lines examined here adenosine-induced 5637 cell death was clearly inhibited by the A_3 adenosine receptor inhibitor MRS1191 and the A_3 adenosine receptor agonist 2-Cl-IB-MECA mimicked the adenosine effect. Furthermore, the adenosine effect was inhibited by knocking-down A_3 adenosine receptor. Collectively, these results indicate that A_3 adenosine receptor mediates 5637 cell apoptosis.

A_3 adenosine receptor is linked to G_i protein involving adenylate cyclase inhibition followed by decreased cAMP production/PKA inhibition and G_q protein involving phospholipase C activation followed by PKC activation [12]. Adenosine-induced 5637 cell death was not affected by the adenylate cyclase activator forskolin, but it was significantly suppressed by the PKC inhibitor GF109203X. This suggests that adenosine induces 5637 cell apoptosis via an A_3 adenosine receptor-mediated G_q protein/PKC activation pathway.

Apoptosis is induced in a caspase-dependent and/or -independent manner. The adenosine effect on 5637 cell death was not inhibited by the pan-caspase inhibitor Z-VAD, suggesting a caspase-independent apoptosis for 5637 cells. Then, the question raised is how A_3 adenosine receptor mediates 5637 cell apoptosis. To address this question, we probed mRNA expression for a variety of apoptosis-related proteins in the real-time RT-PCR analysis. Of them a marked increase in the expression of the AIF mRNA and protein was obtained with adenosine, and the effect was suppressed by knocking-down A_3 adenosine receptor. This interprets that adenosine stimulates the AIF gene transcription and upregulates AIF expression in 5637 cells via A_3 adenosine receptor. Notably, adenosine accumulated AIF in the nucleus by promoting its translocation from the cytosol into the nucleus in 5637 cells. These results, in the light of the fact that AIF induces apoptosis by causing DNA fragmentation in the nucleus in a caspase-independent manner [1-3], indicate that adenosine induces 5637 human bladder cancer cells by upregulating AIF expression and increasing its intranuclear localization, as mediated via A_3 adenosine receptor, dominantly linked to G_q protein. To our knowledge, this is the first showing an apoptotic pathway relevant to A_3 adenosine receptor-mediated AIF upregulation.

Several lines of evidence have pointed to the implication of A_3 adenosine receptor in apoptosis. A_3 adenosine receptor mediates apoptosis in A549 human epithelial lung adenocarcinoma cells by upregulating expression of Bax, Bad, and Puma, to disrupt mitochondrial membrane potentials and to activate caspase-9 followed by the effector caspase-3 [16]. A_3 adenosine receptor mediates apoptosis in Lu-65 human giant cell lung cancers by upregulating p53 expression, to promote p53-dependent Noxa gene transcription, causing activation of caspase-9 in association with disruption of mitochondrial membrane potentials and in turn, caspase-3 [17]. A_3 adenosine receptor also mediates apoptosis in SBC-3 human small cell lung cancer cells by upregulating AMID expression and promoting AMID translocation into the nucleus [18]. A_3 adenosine receptor, alternatively, mediates apoptosis in RCC4-VHL human renal cancer cells by upregulating AMID expression and accumulating AMID in the nucleus [19]. Why diverse signaling pathways underlie A_3 adenosine receptor-mediated apoptosis in a variety of cancer cells, depending upon the cell types, is presently unknown. To address this question, we are currently carrying out further experiments.

In conclusion, the results of the present study show that extracellular adenosine induces caspase-independent apoptosis in 5637 human bladder cancer cells by upregulating AIF expression via an A_3 adenosine receptor, possibly linked to G_q protein, thereby accumulating AIF in the nucleus. This may represent further insight into an A_3 adenosine receptor-mediated apoptotic pathway.

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