Biochemical and Biophysical Research Communications 460 (2015) 759–765

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Adenosine induces apoptosis through TNFR1/RIPK1/P38 axis in colon cancer cells

Shunji Yu [a, b], Daisen Hou [b], Ping Chen [b], Qi Zhang [b], Bin Lv [a], Yunfang Ma [a], Fuchen Liu [c], Hui Liu [c], Evelyn J. Song [d], Dongqin Yang [a, *], Jie Liu [a, *]

[a] Department of Digestive Diseases, Huashan Hospital, Fudan University, Shanghai 200040, China
[b] Institutes of Biomedical Sciences and Department of Immunology of Shanghai Medical School, Fudan University, Shanghai 200032, China
[c] The Third Department of Hepatic Surgery, Eastern Hepatobiliary Surgery Hospital, Second Military Medical University, Shanghai 200438, China
[d] Pennsylvania State University College of Medicine and Hershey Medical Center, Hershey, PA 17033, USA

ARTICLE INFO

Article history:
Received 11 March 2015
Available online 27 March 2015

Keywords:
Adenosine
Apoptosis
RIPK1
p38
TNFR1

ABSTRACT

Adenosine, a metabolite of ATP, ubiquitously exists in a wide range of organs and tissues. We previously reported that adenosine was implicated in apoptosis in many cancer cells by extrinsic and/or intrinsic pathways. Here, we found that adenosine suppresses the cell growth by induction of apoptosis of human colonic cancer cells through a novel mechanism. Adenosine suppresses the cell growth of human SW620 and SW480 colon cells in an adenosine transporter and adenosine kinase dependent manner. Moreover, the cell growth suppression is induced by apoptosis through activation of caspase-3 and PARP, and accumulation of ROS in cells. Importantly, we found that adenosine increases the expression of TNFR1 and RIPK1 and the phosphorylation of p38. Knockdown of TNFR1 or RIPK1 impairs the activation of p38, blocks the cleavage of PARP, and provides partially, yet significantly protection from cell death, including reducing the ROS generation in the colon cancer cells. These results indicate that a TNFR1/RIPK1/P38 axis is present in adenosine-induced apoptosis of colonic cancer cells. This axis triggers apoptosis and plays crucial roles in relay of the death signaling. Our study also provides additional experimental evidence for adenosine as a potent therapeutic drug in cancer therapy.

1. Introduction

The purine nucleoside adenosine, a metabolite of ATP, is omnipresent. Adenosine is generated by breakdown of ATP. It can be further metabolized into inosine and hypoxanthine inside cells [1]. Emerging evidence has indicated the effect of adenosine on the cell death and differentiation [2,3]. Extracellular adenosine induces apoptosis in a variety of cancer cells through two distinct pathways. One is the extrinsic pathway which is associated with four adenosine receptor subtypes, termed A1, A2a, A2b and A3 receptors [4,5]. These G-protein-coupled adenosine receptors display different affinities for adenosine, recruit diverse types of G proteins, and finally trigger distinct downstream signaling pathways in target cells. The other is the intrinsic pathway, which is relevant to adenosine uptake into cells [6]. Adenosine is taken into the cells through adenosine transporters and subsequently becomes converted to AMP, where AMP-activated protein kinase (AMPK) may play a role as a downstream target of AMP [7]. It is reported that adenosine induces apoptosis by the intrinsic pathways in HUH-7 hepatoma cells [7], neuroblastoma [8] and gastric cancer [9]. Adenosine transporter inhibitor (dipyridamole) and adenosine kinase inhibitor (ABT-702) could block adenosine induced apoptosis in those cancer cells [10–12]. So far adenosine is reported to induce apoptosis in many cancer cells. However, it is unknown if and how adenosine induces apoptosis in human colonic cancer cells.

There are over 1 million new cases of colorectal cancer (CRC) and about 500 000 CRC-related deaths annually, which makes CRC the second leading cause of cancer mortality worldwide [13]. Considering the current inefficient treatment of surgical resection, finding an efficient therapeutic strategy for colorectal cancer is crucial. Herein, we report that adenosine suppresses cell growth by induction of apoptosis in SW620 and SW480 colon cancer cell lines and identify a novel mechanism underlying the adenosine-triggered apoptosis in those cells. Our results also provide

* Corresponding authors.
E-mail addresses: kobesakura@163.com (D. Yang), jieliu@fudan.edu.cn (J. Liu).

http://dx.doi.org/10.1016/j.bbrc.2015.03.103
0006-291X/

experimental evidence for the possibility of adenosine in colon cancer therapy.

2. Materials and methods

2.1. Cell line and cell culture

Human colonic cancer cell lines SW620 and SW480 were gifts from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, People's Republic of China). These cells were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum and cultured at 37 °C in a humidified atmosphere with 95% air and 5% CO_2.

2.2. Cell viability assay and live cell tracking in real time

The effects of adenosine on cellular viability and cell growth were determined using CCK8 assay and monitored by IncuCyte™ ZOOM (ESSEN, USA). Briefly, cells were seeded into 96-well plates with 2500 cells and 1600 cells per well, or seeded into 24-well plates with 40 000 cells and 30 000 cells per well for SW620 and SW480, respectively, in triplicate. After overnight incubation, cells were treated with varying concentrations of adenosine (0–3 mM) for indicated hours. The absorbance was then recorded at 450 nm using a micro-plate reader (Tecan Infinite 200 PRO, Switzerland), and the cell growth was monitored in real time by IncuCyte™ ZOOM.

2.3. Cell cycle analysis

Cells were harvested and fixed in 70% ethanol at −20 °C overnight, then stained with propidium iodide (36 mg/ml, Sigma) for 30 min and analyzed by flow cytometry (CyAn ADP, Beckman Coulter, Brea, CA, USA) for cell cycle distribution.

2.4. Apoptosis assay

Adenosine-induced apoptosis was determined by flow cytometry using the Annexin V-FITC Apoptosis Detection Kit following the manufacturer's instructions. Briefly, cells were treated with varying concentrations of adenosine (0–3 mM) for indicated hours. The cells were then harvested, washed in PBS and incubated with Annexin V and propidium iodide (Dojindo) for staining in binding buffer at room temperature for 15 min. The stained cells were analyzed using flow cytometry (CyAn™ ADP, Beckman Coulter, Brea, CA, USA).

2.5. Measurement of intracellular reactive oxygen species (ROS)

The intracellular reactive oxygen species (ROS) was detected using 2′, 7′-dichloro-fluorescein diacetate (DCFH-DA) as a probe, ROS was detected with Reactive Oxygen Species Assay Kit (Beyotime) according to the manufacturer's instructions. Briefly, after treated with varying concentrations of adenosine (0–3 mM) for 72 h, cells were washed twice and loaded with 10 μM DCFH-DA for 20 min at 37 °C. The formation of the fluorescent-oxidized derivative of DCF-DA was monitored using CyAn™ ADP at emission wavelength of 530 nm and excitation wavelength of 485 nm.

2.6. Western blotting

Cell lysates were extracted with cell lysis buffer (Beyotime, China) and the protein concentration in the lysates was quantified using an Enhanced BCA Protein Assay Kit (Beyotime, China). Protein samples with 30–50 μg were loaded for immunoblotting (IB), using appropriate primary antibodies against cleaved PARP, cleaved Caspase-3, RIPK1, TNFR1, p-p38 (Cell Signaling Inc, Danvers, MA, USA), and Actin (Kangwei, China) and horseradish peroxidase-conjugated suitable secondary antibodies, followed by detection with enhanced chemiluminescence (ImageQuant LAS 4000,GE Healthcare, USA).

2.7. siRNA silencing

Colon cancer cells were transfected with siRNA using Lipofectamine® RNAiMAX reagent (Invitrogen, USA) according to the manufacturer's instructions. Briefly, siRNA and Lipofectamine® RNAiMAX were each incubated separately with Opti-MEM for 5 min, mixed together for 15 min at room temperature and then the mixture was applied to the cells plated in 4 ml of medium (final concentration of siRNA is 60 nM). The siRNAs specific for human *TNFR1* and *RIPK1* were purchased from Bioneer (Shanghai, China). The knockdown levels were tested by western blot analysis.

2.8. Statistical analysis

Normally distributed data are shown as mean ± SEM and were subject to Student's t-test to study the relationship between the different variables. For all the tests, $P < 0.05$ was considered statistically significant and three levels of significance ($^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$) were used.

3. Results

3.1. Extracellular adenosine inhibited the growth of colon cancer cells

To investigate the effect of extracellular adenosine on SW620 and SW480 cell lines, cells were treated with various concentrations of adenosine at indicated hours and the cell viability was determined using the CCK8 assay and their growth curve was monitored in real-time by the IncuCyte™ live-cell imaging system. Treatment with adenosine for 72 h significantly suppressed the growth of SW620 and SW480 cells as compared to that of the untreated control group (Fig. 1A and B). With the CCK8 assay, it was shown that extracellular adenosine reduced SW620 and SW480 cell viability in a dose (0.3–3 mM) and time (24–72 h) dependent manner (Fig. 1C and D). Treatment with 3 mM or 1 mM adenosine for 72 h reduced cell viability to nearly 40% of control group, in both SW620 and SW480 cells. Adenosine treatment with medium-to-high dose (1–3 mM) could lead both SW620 and SW480 cells to the characteristic apoptotic morphological change, including cell shrinkage in shape and in size; but control cells did not show the similar morphological change (Fig. 1E and F). In addition, adenosine-induced decrease of cell viability was significantly blocked by dipyridamole, an adenosine transporter inhibitor (Supplementary Fig. 1A and B), and ABT-702, an inhibitor of adenosine kinase (Supplementary Fig. 1C and D) in a dose-dependent manner. These data indicated that adenosine was transported into the cells and converted to AMP by adenosine kinase, and subsequently induces cell death.

3.2. Extracellular adenosine prompted G2/M arrest

To understand the mechanism underlying the adenosine-induced growth arrest in colonic cancer cells, the cell cycle profile in adenosine-treated SW620 and SW480 cells was initially explored. As shown in Supplementary Fig. 2A and B, a dose dependent G2/M phase arrest was observed in the cells upon

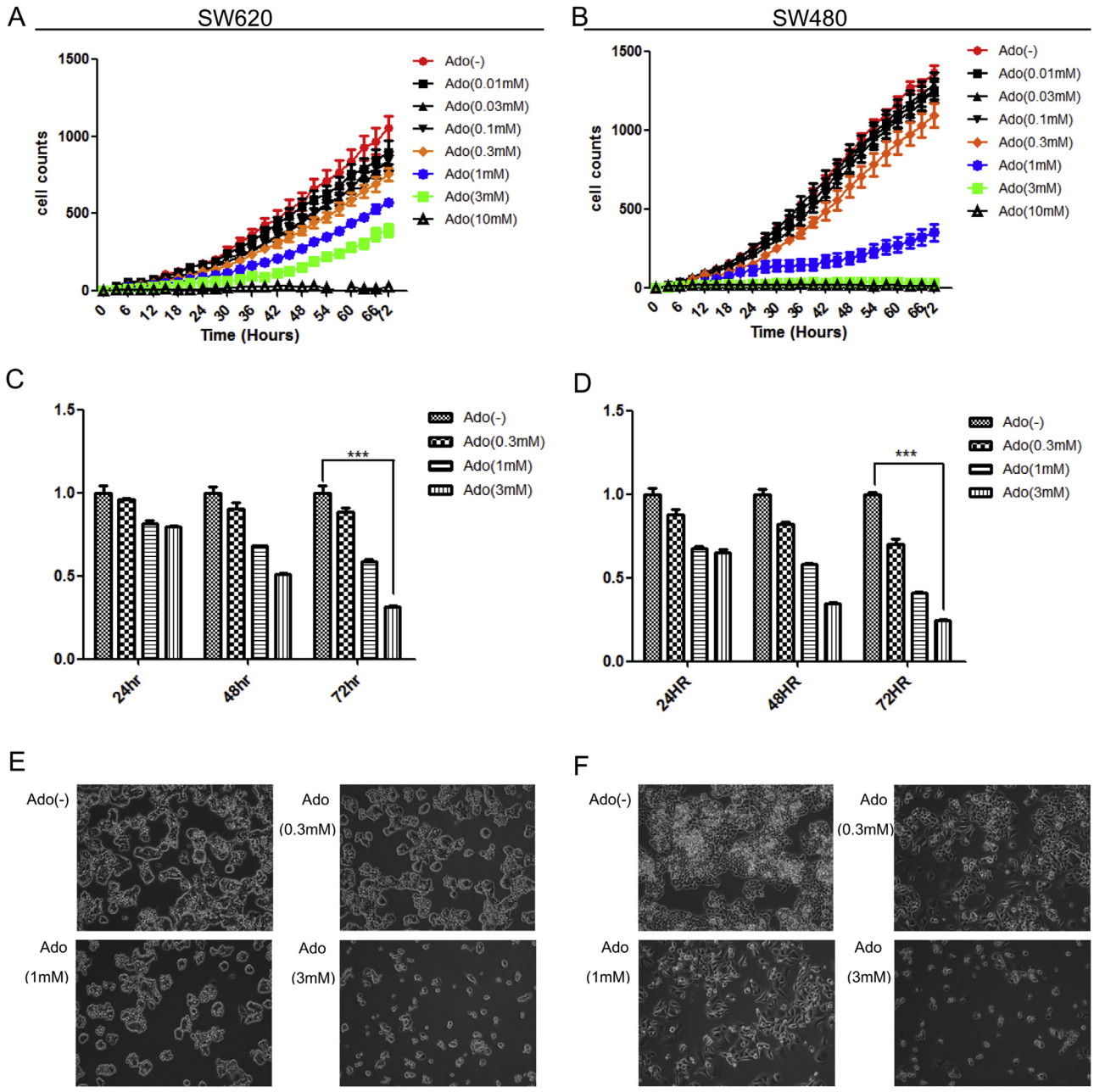

Fig. 1. Effect of adenosine on growth arrest of colonic SW620 and SW480 cancer cells. A–B: SW620 (A) and SW480 (B) were treated with adenosine (0–10 mM) and monitored in real time by the live-cell imaging system IncuCyte™. Representative growth curves of cells treated with adenosine at the indicated concentration were shown. Each curve was performed at least four times, and each time point was determined in triplicate. C–D: SW620 (C) and SW480 (D) cells treated with adenosine at the indicated concentrations for 24–72 h were quantified for cell viability by CCK8 assay. Each column represents the mean ± SEM values from four replicate wells of three separate experiments. (***P < 0.001). E–F: Cellular morphological observation of SW620 (E) and SW480 (F) treated with adenosine at the indicated concentrations for 72 h.

adenosine treatment. Adenosine-induced G2/M phase arrest occurred at 24 h and reached the peak at 72 h. The statistical analysis showed that the percentage of G2/M phase arrest was significantly higher in the two cell lines treated with adenosine (1 and 3 mM) for 72 h than in control cells (Supplementary Fig. 2C and D).

3.3. Extracellular adenosine induced apoptosis

Next, the apoptotic effect of adenosine on SW620 and SW480 was explored with flow cytometry using Annexin V-FITC/PI staining, a classic apoptosis assay. The result showed that the proportion of PI (+) and Annexin V (+) cells, corresponding to apoptosis, was increased proportionally to the adenosine dose (Fig. 2A and B). The percentage of apoptotic cells significantly increased from 4.0 ± 0.1% in untreated SW620 cells to 18.4 ± 0.8% in the cells treated with 3 mM adenosine ($P < 0.001$) (Fig. 2C). More obviously in SW480 cells, the percentage of apoptotic cells boosted from 6.2 ± 0.6% in non-treatment to 71.2 ± 0.3% in treatment group with 3 mM adenosine for 72 h ($P < 0.001$) (Fig. 2D). These data suggested that adenosine induced the apoptosis in SW620 and SW480 cells. Moreover, the level of cleaved caspase-3 (c-caspase-3) and the expression of the caspase-3 downstream target (cleaved poly ADP ribose polymerase, c-PARP) were explored in SW620 and SW480

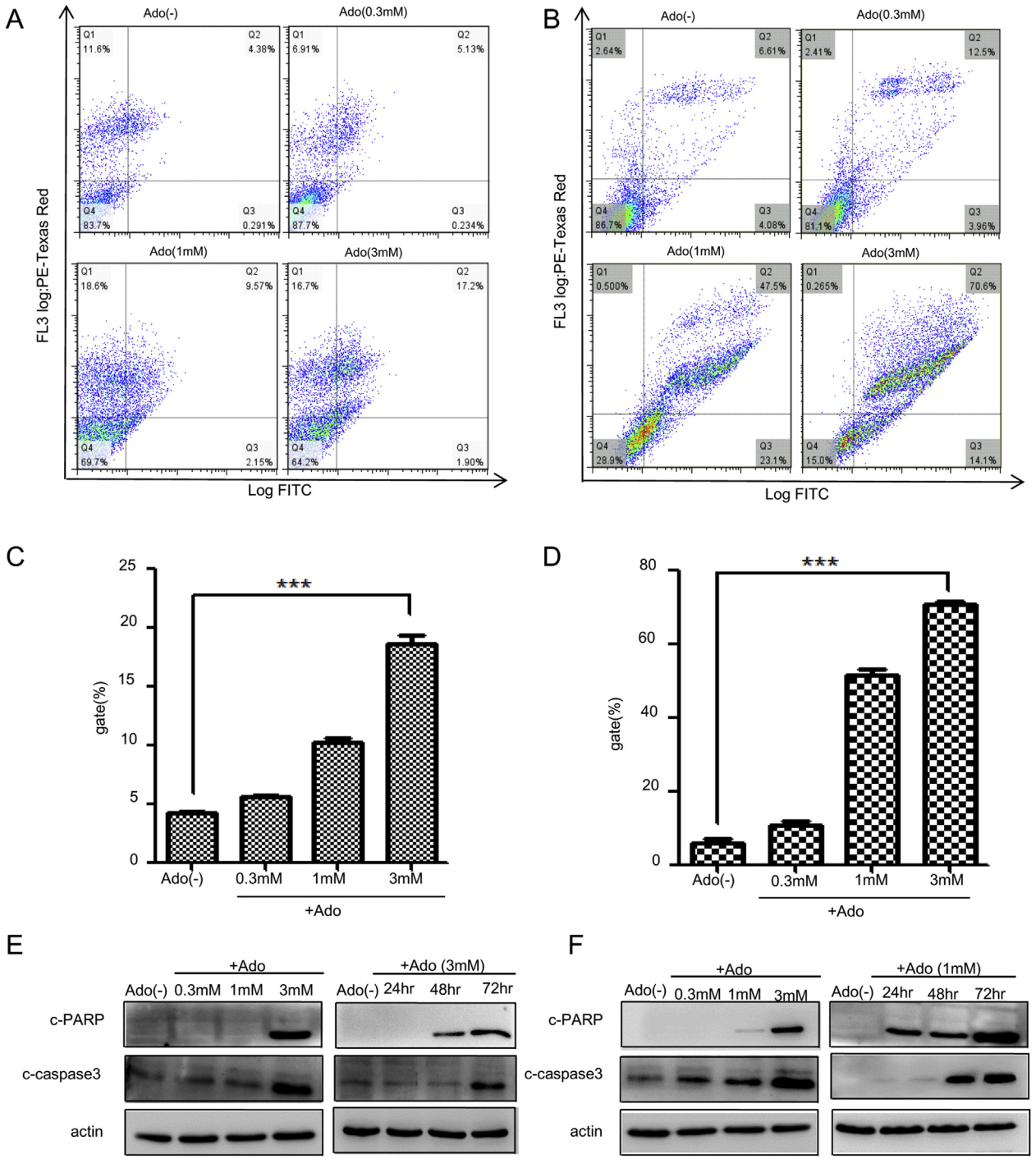

Fig. 2. Extracellular adenosine induced apoptosis in SW620 and SW480 cells. A–B: SW620 (A) and SW480 (B) cells were treated with adenosine (0–3 mM) for 72 h and harvested for apoptosis assay by flow cytometry using PI and Annexin V-FITC double staining. C–D: Each graph represents the percentage of PI positive and Annexin V positive cells in adenosine-treated SW620 (C) and SW480 (D) cells. The results are means ± SEM of three independent experiments. (***$P < 0.001$). E–F: SW620 (E) and SW480 (F) cells were treated with adenosine as indicated and were subjected to western blot using the indicated antibodies.

cells upon adenosine treatment. A time and dose-dependent increase of c-caspase-3 and c-PARP were clearly observed in SW620 treated with 3 mM adenosine and in SW480 cells treated with 1 mM adenosine for 72 h (Fig. 2E and F). These data further confirmed that adenosine induced the cellular apoptosis. Taken together, our data indicated that adenosine-induced cell growth suppression in SW620 and SW480 was due to G2/M phase arrest and apoptosis.

3.4. Extracellular adenosine elicited p38 activation and ROS production

To further elucidate the molecular mechanism underlying the adenosine-induced apoptosis, the activation of death receptor signaling pathway were examined by Western blot. A sustained increment of phospho-p38 (p-p38) and protein level of TNFR1 and RIPK1 were observed in both SW620 and SW480 upon various

doses of adenosine treatment (Fig. 3A), implicating death receptors in the adenosine-mediated cell death. To further explore whether prolonged phosphorylation of MAPK contributed to apoptosis [14], the effect of SB203580, an efficient p38 inhibitor, on adenosine-induced decrease of cell viability was explored in cells. The results showed that SB203580 partially and significantly rescued the adenosine-induced decrease of cell viability in SW620 co-treated with 3 mM adenosine and in SW480 cells with 1 mM adenosine for 72 h in a dose dependent manner. These results indicated that inhibition of MAPK pathway shielded SW620 and SW480 cells from adenosine-induced cell death (Fig. 3B).

Our previous studies have reported that ROS level is heightened in adenosine-triggered apoptosis, which mediates the mitochondrial dysfunction in hepatocarcinoma cells [15]. We examined the effect of adenosine on intracellular ROS levels through flow cytometry analysis. As shown in Fig. 3C, the ROS level raised with the increase of adenosine dose in both cell lines. The exposure of SW620 cells to 3 mM adenosine for 72 h led to a 20-fold accumulation of intracellular ROS, compared to that of control group (Fig. 3D). A similar increased ROS generation occurred in SW480 cells (Fig. 3E).

3.5. Silence of TNFR1/RIPK1 lessened apoptosis

It is well known that ligation of TNFR1 recruits the adaptor molecule TRADD, which in turn binds FADD and caspase-8, and the dimerization of the complex triggers its proteolytic activity, promoting apoptosis [16]. In addition, recent studies reveal that RIPK1, independent of its kinase activity, has a scaffold function that contributes to activation of MAPK following ligation of TNFR1 [16,17].

In order to further explore the role of TNFR1 and RIPK1 in adenosine-induced apoptosis, a RNA interference approach was used to silence the TNFR1 or RIPK1. We found that knockdown of TNFR1 remarkably reduced cleavage of PARP and phosphorylation of p38 in SW620 and SW480 (Fig. 4A). A similar effect was also observed in both cells by abrogation of RIPK1 (Fig. 4B). CCK8 assay revealed that silencing either TNFR1 or RIPK1 notably blocked the adenosine-induced decrease of cell viability in the two cell lines. Abrogation of TNFR1 or RIPK1 increased cell viability from 32.8 ± 0.3% to 45.3 ± 1.4% or to 54.1 ± 0.8% in SW620 with 3 mM adenosine treatment (Fig. 4C), and from 43.0 ± 0.7% to 54.4 ± 1.2% or to 63.1 ± 2.4% in SW480 with 1 mM adenosine treatment (Fig. 4D). Similarly, silencing either TNFR1 or RIPK1 significantly blocked adenosine-induced apoptosis and ROS generation. TNFR1 or RIPK1 knockdown declined adenosine-induced apoptotic proportion to nearly half of that with indicated concentration of adenosine treatment alone in both SW620 (Fig. 4E) and SW480 (Fig. 4F) cells. In addition, blockade of TNFR1 or RIPK1 attenuated the adenosine-induced ROS generation from 33.2 ± 0.8% to 17.3 ± 1.3% and to 15.4 ± 2.1%, respectively, in SW620 (Fig. 4G) and from 20.3 ± 0.7% to 10 ± 1.3% and to 8.1 ± 0.3% in SW480 (Fig. 4H). These data indicated that p38 inhibitor and silencing of TNFR1 or RIPK1 partially, yet significantly protected SW620 and SW480 cells from adenosine-induced cell death. Taken together, these results clearly demonstrated that adenosine-induced cell apoptosis was mediated through TNFR1/RIPK1/p38 axis in cells.

4. Discussion

In this study, we observed that adenosine significantly induces cell growth suppression and G2/M phase arrest in colon cancer cells. Inhibition of adenosine transporter and the adenosine kinase rescued the adenosine-induced cell growth arrest, suggesting that adenosine induces cell death through intrinsic pathways, i.e. its uptake into cells and conversion to AMP are required to exert this effect. We further clearly demonstrated that adenosine significantly increased apoptosis, induced activation of caspase-3 and PARP, and increased ROS generation, the byproduct of cell death, in colon cancer cells. Importantly, we observed adenosine induced the

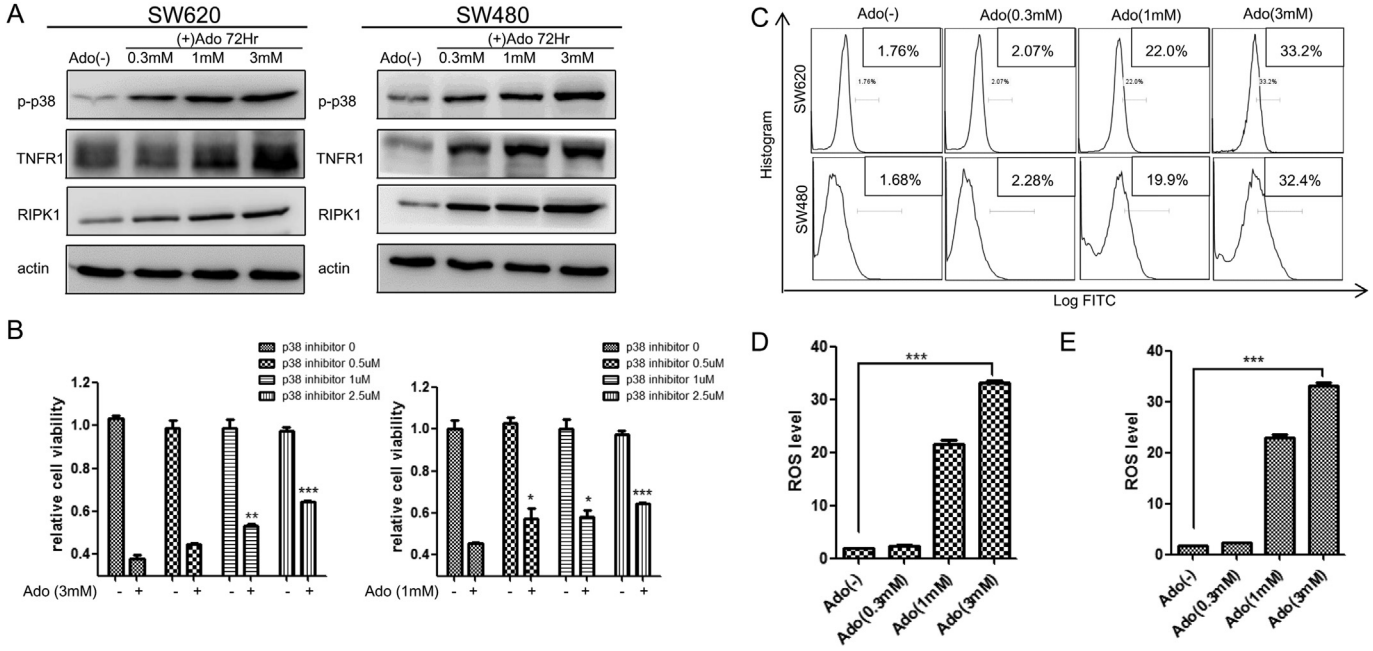

Fig. 3. p38 was required and ROS was the byproduct implicated in the adenosine-triggered cell death. (A) SW620 and SW480 cells were treated with adenosine (0–3 mM) for 72 h and cell lysates were prepared and analyzed by western blot using the indicated antibodies. (B) SW620 and SW480 cells co-treated with adenosine and p38 inhibitor, SB203580, as indicated were quantified for cell viability by CCK8 assay. Each column represents the mean ± SEM of three independent experiments. (***P < 0.001). (C) SW620 and SW480 cells were treated with different concentrations of adenosine for 72 h. Intracellular ROS levels were determined using DCFH-DA. D–E: The quantitated data of ROS levels were shown in the bar figure for SW620 (D) and SW480 (E) cells. The results are presented as mean value ± SEM from three independent experiments. (***P < 0.001).

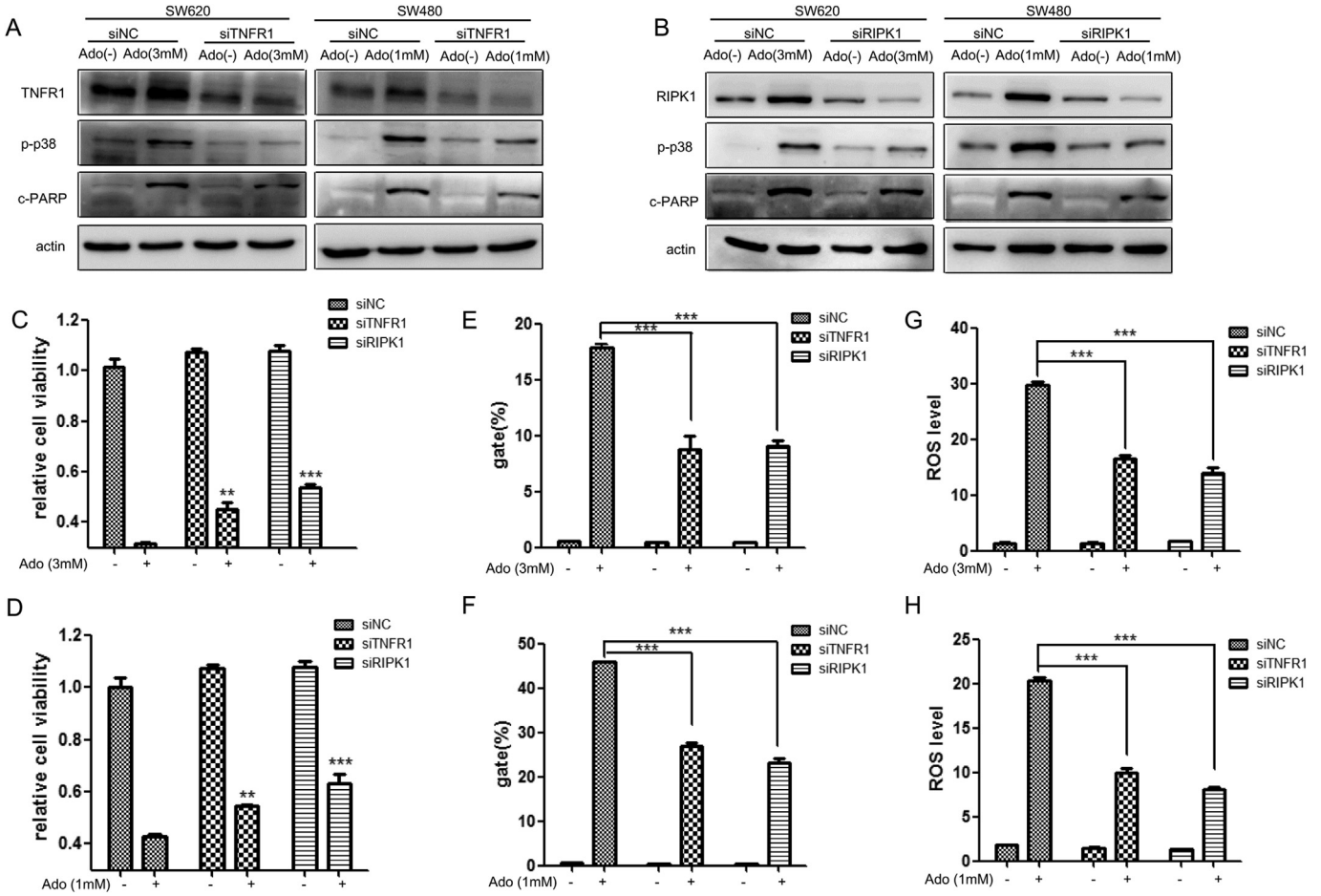

Fig. 4. Blockage of death receptor signaling pathway attenuated apoptosis. The siTNFR1or siRIPK1 transfected cells were treated with adenosine before western blot analysis (A) and (B). The cell viability was quantitated in SW620 (C) and SW480 (D) by CCK8 assay. The percentage of apoptotic cells in SW620 (E) and SW480 (F) cells. The intracellular ROS levels in SW620 (G) and SW480 (H). The results are displayed as mean ± SEM value from three independent experiments. (***P < 0.001).

sustained phosphorylation of p38 and p38 inhibitor attenuated the adenosine-induced cell death. We also found that adenosine increased the expression of TNFR1 and RIPK1, the components of death receptor signaling, and that knockdown of either TNFR1 or RIPK1 resulted in a significant decrease in the phosphorylation of p38 and the cleavage of PARP and additionally partially rescued adenosine-induced cell death. These data suggest that adenosine induces apoptosis through TNFR1/RIPK1/P38 axis in colon cancer, which is a novel mechanism for the adenosine-triggered cell death. Collectively, we proposed a novel model of the adenosine-induced apoptosis in colon cancer cells (Supplementary Fig. 3).

Adenosine induced apoptosis in colon cells by entering into cell, which reinforces our previous report in hematoma cells [7]. However, in colonic cancer cells, adenosine activated capspase-3 by increasing the TNFR1 and RIPK1 expression and activation of p38. This is different from the effect of adenosine in hepatoma cells, which mainly down-regulates the FLIP expression and activates caspase-8 cleavage. Adenosine is reported to induce apoptosis by intrinsic pathways by regulating the transcription of apoptosis-mediator genes in cancer cells [19]. Therefore, we considered that adenosine increased the expression of TNFR1 and RIPK1 by enhancing their transcription in cells although the detailed mechanisms need to be further clarified.

Death receptor TNFR1 is a trimeric cytokine receptor, which triggers the recruitment of other adaptors including TRADD and RIPK1 to determine the outcome of the response [16]. Receptor interacting protein kinase-1 (RIPK1) represents an important signaling node [18], which responds to a variety of input signals, involving ligands for Toll-like receptors (TLRs), those elicited by TNF family cytokines, viral infection and interferons. Although new properties of RIPK1 signaling are increasingly emerging, the signaling network mastered by RIPK1 mainly controls the activation of MAPK and NF-kB, and both of them are responsible for the cell death or survival. In this study, knockdown of either TNFR1 or RIPK1 partially rescued the cell viability loss which was confirmed by the Annexin V-FITC/PI staining assay, and decreased the aggregation of the byproduct ROS. Apart from the delicate cleavage of PARP, the blockade of death receptors resulted in a significant decrease in the phosphorylation of p38. These data indicated that adenosine induced the increase of TNFR1 and RIPK1, which further resulted in the activation of p38. This TNFR1/RIPK1/p38 axis is responsible for triggering apoptosis in the colonic cancer cells.

Activation of MAPK pathways is documented to be implicated in ROS generation, caspases activation and PARP cleavage in colon cancer. It is reported that non-aromatic B-ring flavonoid (DHEC) induces apoptosis of human colon HT-29 tumor cell through generation of ROS as well as activation of MAPK signaling pathway [20]. Another drug (Ilimaquinone) induces the death receptor expression and sensitizes the colon cancer cells to apoptosis by activation of ROS-ERK/p38 MAPK-CHOP signaling pathways [21]. It is also reported that drug (Gingerol) induces apoptosis through activation of caspase-8, -9, -3 & -7 and cleavage of PARP, which is accompanied

by phosphorylation of the mitogen-activated protein kinase (MAPKs) family, c-Jun N-terminal kinase (JNK), p38 MAPK (p38), and extracellular signal-regulated kinase (ERK) [22].

Our data demonstrate that p38 inhibitor could block adenosine-induced cell death in colonic cells, which supports previous studies and indicates the role of p38 activation in the signal cascade from the death receptor of adenosine-induced apoptosis in cells.

In summary, adenosine suppresses the growth of human colonic cancer cells by induction of apoptosis. Adenosine which is transported into the cells and converted to AMP triggers apoptosis by activation of caspase-3 and PRAP, and also results in the accumulation of ROS. Furthermore, activation of TNFR1/RIPK1/p38 axis triggers adenosine-caused apoptosis and plays crucial roles in relaying the death signaling in colon cancer cells.

Conflict of interest

None.

Acknowledgment

This work is supported by National Natural Science Foundation of China to Jie Liu (81328021) and Dongqin Yang (81372652), the Ministry of Science and Technology of China to Jie Liu (No. 2012CB910104), and one hundred person project of the Shanghai Health to Hui Liu (XBR2013117). We thank Melanie Hartman and Evelyn J. Song for their proof reading for English grammar.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.03.103

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.03.103

References

[1] H. Zimmermann, Extracellular metabolism of ATP and other nucleotides, Naunyn. Schmiedebergs Arch. Pharmacola 362 (2000) 299–309.
[2] L. Antonioli, P. Pacher, E.S. Vizi, G. Haskó, CD39 and CD73 in immunity and inflammation, Trends Mol. Med. 19 (2013) 355–367.
[3] H.K. Eltzschig, Adenosine: an old drug newly discovered, Anesthesiology 111 (2009) 904–915.
[4] S. Gessi, S. Merighi, V. Sacchetto, C. Simioni, P.A. Borea, Adenosine receptors and cancer, Biochim. Biophys. Acta 1808 (2011) 1400–1412.
[5] S. Sheth, R. Brito, D. Mukherjea, L.P. Rybak, V. Ramkumar, Adenosine receptors: expression, function and regulation, Int. J. Mol. Sci. 15 (2014) 2024–2052.
[6] A. dos Santos-Rodrigues, J.M. Ferreira, R. Paes-de-Carvalho, Differential adenosine uptake in mixed neuronal/glial or purified glial cultures of avian retinal cells: modulation by adenosine metabolism and the ERK cascade, Biochem. Biophys. Res. Commun. 414 (2011) 175–180.
[7] D. Yang, T. Yaguchi, T. Nakano, T. Nishizaki, Adenosine activates AMPK to phosphorylate Bcl-XL responsible for mitochondrial damage and DIABLO release in HuH-7 cells, Cell. Physiol. Biochem. Int. J. Exp. Cell. Physiol. Biochem. Pharmacol. 27 (2011) 71–78.
[8] M. Hashemi, F. Karami-Tehrani, S. Ghavami, S. Maddika, M. Los, Adenosine and deoxyadenosine induces apoptosis in oestrogen receptor-positive and -negative human breast cancer cells via the intrinsic pathway, Cell Prolif. 38 (2005) 269–285.
[9] M. Saitoh, K. Nagai, K. Nakagawa, T. Yamamura, S. Yamamoto, T. Nishizaki, Adenosine induces apoptosis in the human gastric cancer cells via an intrinsic pathway relevant to activation of AMP-activated protein kinase, Biochem. Pharmacol. 67 (2004) 2005–2011.
[10] K. Sai, D. Yang, H. Yamamoto, H. Fujikawa, S. Yamamoto, T. Nagata, et al., A(1) adenosine receptor signal and AMPK involving caspase-9/-3 activation are responsible for adenosine-induced RCR-1 astrocytoma cell death, Neurotoxicology 27 (2006) 458–467.
[11] N.M. Elsherbiny, S. Ahmad, M. Naime, A.M. Elsherbini, S. Fulzele, M.M. Al-Gayyar, et al., ABT-702, an adenosine kinase inhibitor, attenuates inflammation in diabetic retinopathy, Life Sci. 93 (2013) 78–88.
[12] Y. Yasuda, M. Saito, T. Yamamura, T. Yaguchi, T. Nishizaki, Extracellular adenosine induces apoptosis in Caco-2 human colonic cancer cells by activating caspase-9/-3 via A(2a) adenosine receptors, J. Gastroenterol. 44 (2009) 56–65.
[13] S. Haraldsdottir, H.M. Einarsdottir, A. Smaradottir, A. Gunnlaugsson, T.R. Halfdanarson, Colorectal cancer - review, Læknablaðið 100 (2014) 75–82.
[14] W.-X. Zhao, S.-S. Tang, X. Jin, C.-M. Zhang, T. Zhang, C.-C. Wang, et al., Olaquindox-induced apoptosis is suppressed through p38 MAPK and ROS-mediated JNK pathways in HepG2 cells, Cell Biol. Toxicol. 29 (2013) 229–238.
[15] Y. Ma, J. Zhang, Q. Zhang, P. Chen, J. Song, S. Yu, et al., Adenosine induces apoptosis in human liver cancer cells through ROS production and mitochondrial dysfunction, Biochem. Biophys. Res. Commun. 448 (2014) 8–14.
[16] H. Hsu, J. Xiong, D.V. Goeddel, The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation, Cell 81 (1995) 495–504.
[17] C.-K. Ea, L. Deng, Z.-P. Xia, G. Pineda, Z.J. Chen, Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO, Mol. Cell 22 (2006) 245–257.
[18] R. Weinlich, D.R. Green, The two faces of receptor interacting protein kinase-1, Mol. Cell 56 (2014) 469–480.
[19] D. Yang, T. Yaguchi, C.-R. Lim, Y. Ishizawa, T. Nakano, T. Nishizaki, Tuning of apoptosis-mediator gene transcription in HepG2 human hepatoma cells through an adenosine signal, Cancer Lett. 291 (2010) 225–229.
[20] A. Wei, D. Zhou, C. Xiong, Y. Cai, J. Ruan, A novel non-aromatic B-ring flavonoid: isolation, structure elucidation and its induction of apoptosis in human colon HT-29 tumor cell via the reactive oxygen species-mitochondrial dysfunction and MAPK activation, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 49 (2011) 2445–2452.
[21] M.T. Do, M. Na, H.G. Kim, T. Khanal, J.H. Choi, S.W. Jin, et al., Ilimaquinone induces death receptor expression and sensitizes human colon cancer cells to TRAIL-induced apoptosis through activation of ROS-ERK/p38 MAPK-CHOP signaling pathways, Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 71 (2014) 51–59.
[22] M.J. Ryu, H.S. Chung, [10]-Gingerol induces mitochondrial apoptosis through activation of MAPK pathway in HCT116 human colon cancer cells, In Vitro Cell. Dev. Biol. Anim 51 (2015) 92–101.