Original Article

Adenanthin inhibits pancreatic cancer proliferation by regulation of H_2O_2/ROS

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Background: Adenanthin, a diterpenoid isolated from the leaves of Rabdosia adenantha, has anti-tumor activities. However, its role and mechanism in pancreatic cancer are unclear. This study aims to elucidate the mechanism of adenanthin induced death of pancreatic cancer cells by regulating hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS) levels.

Methods: Adenanthin was used to detect its cell activity on pancreatic cancer cells (Aspc-1) using the Cell Counting Kit-8 (CCK-8) and colony forming assays. Hoechst 33258 fluorescence staining and flow cytometry related experiments were used to detect its impact on apoptosis and cell cycle of Aspc-1 cells. Western blot was used to detect the expression of cycle and apoptosis related proteins, and the concentration of H_2O_2 and ROS in Aspc-1 cells were measured by content determination kit. A mouse tumor transplantation model was established and the expression of related proteins after administration of adenanthin was detected.

Results: Adenanthin can inhibit the proliferation and induce apoptosis of pancreatic cancer cells. The results of propidium iodide (PI) single staining flow cytometry showed that adenanthin significantly blocked Aspc-1 cells in the S phase and G2/M phase. Further exploration of its mechanism found that adenanthin significantly increased the content of H_2O_2 and ROS in cells, and realized the inhibitory effect on pancreatic cancer cells by regulating apoptosis and cyclin. The transplanted tumor model in mice was consistent with the results of cell experiments.

Conclusions: In pancreatic cancer, adenanthin can significantly increase the content of H_2O_2 and ROS, induce apoptosis and cycle arrest of pancreatic cancer cells, and ultimately play a role in treating pancreatic cancer. Therefore, adenanthin is expected to become a new drug against pancreatic cancer.

Keywords: Adenanthin; hydrogen peroxide (H_2O_2); reactive oxygen species (ROS); pancreatic cancer

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Introduction

Pancreatic cancer is a highly malignant disease that is characterized by rapid progression and poor prognosis (1,2). To date, the therapeutic efficacy of drugs against pancreatic cancer has been unsatisfactory, and the development of innovative anti-pancreatic cancer drugs has encountered significant obstacles. Therefore, it is of great significance to conduct in-depth research on the mechanisms of the occurrence and development of pancreatic cancer, to discover new proteins that have significant impact on

pancreatic cancer, and to provide new targets for the development of innovative anti-pancreatic cancer drugs.

Adenanthin is a diterpenoid compound extracted from the herb Rabdosia adenantha that has anti-inflammatory and anti-tumor activities (3). Previous study (4) found that adenanthin can induce intracellular reactive oxygen species (ROS) accumulation by inhibiting the activity of peroxiredoxin (Prx) I/II, ultimately leading to the killing of liver cancer cells. Multiple studies have also confirmed the specific targeting of Prx by adenanthin and its inhibitory effect on its activity (4,5), and have used it as a natural inhibitor of Prx for related mechanistic studies (6-8). However, so far, there have been no reports on the inhibitory effect of adenanthin on pancreatic cancer cells. It has only been reported that Prx I is highly expressed in pancreatic cancer tissues and is significantly associated with the prognosis of pancreatic cancer; increased expression of Prx I is associated with poorer prognosis (9). The Prx family is considered to be the most important enzyme for decomposing hydrogen peroxide (H_2O_2) in the body. Current views suggest that Prx can regulate ROS by controlling the level of H_2O_2, thereby reducing oxidative stress, decreasing tumor cell apoptosis, promoting proliferation, and on the other hand, can directly activate tumor growth signals by regulating ROS, thereby promoting tumor proliferation and metastasis (10-12).

Adenanthin, as a natural inhibitor of Prx, exerts significant effects on the proliferation and apoptosis of pancreatic cancer through the regulation of H_2O_2/ROS but its specific mechanisms has not been reported. Therefore, this study focuses on adenanthin to investigate its regulatory effects on the proliferation of pancreatic cancer cells and its potential mechanisms, with the aim of adenanthin becoming a new drug for anti-pancreatic cancer. We present this article in accordance with the MDAR and ARRIVE reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-874/rc).

Highlight box

Key findings

- Adenanthin can make the content of hydrogen peroxide (H_2O_2) exceed the endurance limit of pancreatic cancer cells by regulating the content of H_2O_2, thus leading to DNA damage, cycle arrest and apoptosis of pancreatic cancer cells, and ultimately leading to the death of pancreatic cancer cells.

What is known and what is new?

- Previous studies have found that adenanthin can inhibit the activity of peroxiredoxin (Prx) I/II, leading to the accumulation of intracellular reactive oxygen species (ROS) and ultimately killing liver cancer cells. However, there have been no reports on the inhibitory effect of adenanthin on pancreatic cancer cells.
- In this study, adenanthin can inhibit the proliferation and induce apoptosis of pancreatic cancer cells both *in vivo* and *in vitro*.

What is the implication, and what should change now?

- Adenanthin is expected to become a new drug against pancreatic cancer.

Methods

Experimental cells and tissue specimens

The human pancreatic cancer cell line Aspc-1 was purchased from the Cell Bank of the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Cancer adjacent tissue and cancer tissue specimens from six pancreatic cancer patients were obtained from surgeries at Suzhou Kowloon Hospital. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Suzhou Kowloon Hospital (approval number: Kowloon Ethics Review KY-2022-001), and informed consent was obtained from all the patients.

Major reagents and instruments

Adenanthin was purchased from Shanghai Biotech Pharmaceutical Technology Co., Ltd. (Shanghai, China); Cell Counting Kit-8 (CCK-8) and Hoechast 33258 reagents were purchased from Shanghai Biyun Tian Biotechnology Co., Ltd. (Shanghai, China); TRIzol was purchased from Invitrogen (USA); cell cycle assay kit and apoptosis assay kit were purchased from Jiangsu Kaiji Biotechnology Co., Ltd. (Nanjing, China); enzyme linked immunosorbent assay (ELISA) kits were purchased from Hangzhou Union-Biotechnology Co., Ltd. (Hangzhou, China); all antibodies were purchased from Cell Signaling Technology (USA); polymerase chain reaction (PCR) primers were synthesized by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China).

The Western blot electrophoresis system was purchased from Bio-Rad (USA); the multifunctional enzyme labeling instrument was purchased from Thermo Fisher (USA).

Experimental methods

CCK-8 assay for cell proliferation

Aspc-1 cells were seeded in a 96-well plate at a density of

2,000 cells/well and treated with different concentrations of adenanthin (5, 1, 0.5, 0.1, 0.05, 0.01, 0 μM) for 1, 2, 3, and 5 days (with medium change every two days). Then, 20 μL of CCK-8 solution was added to each well, followed by 1 hour of incubation. Absorbance at 450 nm was measured using a microplate reader to indirectly assess cell viability.

Colony formation assay

Cells were seeded in a 6-well plate at a density of 1,000 cells/well and treated with different concentrations of drugs (0, 0.1, 0.5, 1 μM) the next day. After 14 days of incubation, colonies were fixed, stained with 0.5% crystal violet (Sigma Aldrich, USA), and counted under a microscope. Colony inhibition rate was calculated.

Annexin-V assay for apoptosis detection

Aspc-1 cells in logarithmic growth phase were treated with different concentrations of adenanthin (1, 0.5, 0 μM) for 48 hours. Annexin-V and propidium iodide (PI) staining were performed, followed by flow cytometry analysis.

Hochest 33258 staining

Aspc-1 cells were treated with different concentrations of adenanthin (1, 0.5, 0 μM) for 48 hours, fixed, stained with Hochest 33258, and observed under a fluorescence microscope.

Cell cycle analysis

Aspc-1 cells were treated with different concentrations of adenanthin (1, 0.5, 0 μM) for 48 hours, fixed, stained, and analyzed using flow cytometry.

ELISA for H2O2 and ROS detection

Aspc-1 cells were treated with different concentrations of adenanthin (1, 0.5, 0 μM) for 24 hours, and the levels of H_2O_2 and ROS in the cells were measured according to the ELISA kit instructions.

Tumor xenograft mouse model

The nude mice (~20 g, 4 weeks, male) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All mice were maintained in a Specific-Pathogen-Free facility (Grade = Nasal-1000) and subcutaneously injected with Aspc-1 cells. Mice with tumor volumes ranging from 100 to 300 mm^3 were selected for subsequent grouping and drug experiments. A protocol was prepared before the study without registration. Mice were divided into three groups, with 10 mice in each group: control group receiving saline, low-dose adenanthin group (10 mg/kg/day), and high-dose adenanthin group (30 mg/kg/day). Treatment was administered via intraperitoneal injection for 20 days, with tumor volume and mouse weight measured every two days. After 21 days, mice were euthanized, tumors were collected, and protein expression was analyzed by Western blot. All animal experiments involved in this study were approved by the Animal Experimentation Ethics Committee at Genepharma Company (No. IACUC-2022539), in compliance with institutional guidelines for the care and use of animals.

Western blot

Aspc-1 cells in logarithmic growth phase were harvested and seeded into a 96-well plate, then treated with different concentrations of adenanthin (1, 0.5, 0.1, 0 μM) for 48 hours. After incubation, cellular proteins were collected. The bicinchoninic acid (BCA) protein assay kit was utilized to quantify the protein content within the cells. Proteins were separated by Sodium Dodecyl Sulfate (SDS)-polyacrylamide gel electrophoresis and subsequently transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were incubated overnight at 4 °C with primary antibodies, followed by incubation with secondary antibodies at room temperature for 1 hour. Protein bands were detected using an enhanced chemiluminescence (ECL) system.

Statistical analysis

Data analysis was performed using GraphPad Prism 7 software. All experiments were independently repeated three times, and results were expressed as mean ± standard deviation (SD). Homogeneity of variance was tested, and if the data met the assumptions of normal distribution and homogeneity of variance, one-way analysis of variance (ANOVA) was employed, followed by least significant difference-t (LSD-t) test for pairwise comparisons. If variance homogeneity was not met, non-parametric methods were used. The significance level was set at α=0.05, denoted by * for P<0.05, ** for P<0.01, *** for P<0.001, and **** for P<0.0001.

Results

Expression of Prx I in pancreatic cancer tissues is significantly higher than adjacent tissues

Tissues from six pancreatic cancer patients, including

cancer adjacent tissues and cancer tissues, were collected for Western blot analysis to detect the expression of Prx I. The results showed a significant increase in the expression of Prx I in pancreatic cancer tissues compared to adjacent tissues (*Figure 1*). This suggests that the elevated expression of Prx I may be associated with the prognosis of pancreatic cancer, consistent with previous reports, indicating the potential involvement of Prx I in the proliferation of pancreatic cancer.

Inhibition of Aspc-1 cell proliferation by adenanthin

We subsequently used the reported small molecule inhibitor of Prx I, adenanthin, as a probe to test its activity on pancreatic cancer cells (Aspc-1). Aspc-1 cells were seeded at a density of 2,000 cells/well in a 96-well plate and treated with different concentrations of adenanthin (20, 10, 5, 1, 0.5, 0.1, 0.05, 0.01, 0 μM) for 1, 2, 3 days, and 1 μM adenanthin was used to treat cells for 1, 2, 3, 5 days, followed by CCK-8 assays to measure cell viability. The results showed that adenanthin could significantly inhibit cell proliferation in a time- and dose-dependent manner (*Figure 2A*).

To further validate the antitumor effect of adenanthin, we performed a colony formation assay. Based on the above experimental results, we selected concentrations of 0.1, 0.5, and 1 μM adenanthin to treat Aspc-1 cells. Cells were seeded at a specific concentration in a 6-well plate and allowed to grow overnight, followed by treatment with different drugs the next day and continued incubation in the culture chamber for 14 days, after which fixation and staining were performed. The results revealed that adenanthin significantly inhibited the proliferation of pancreatic cancer cells, significantly reduced the growth of pancreatic cancer cells, and the colony formation rate of cells treated with 1 μM adenanthin was as low as 1%. These results indicate that adenanthin can significantly inhibit the proliferation of pancreatic cancer cells (*Figure 2B*).

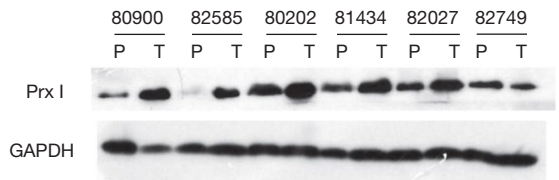

Figure 1 The expression of Prx I in pancreatic cancer was significantly higher than that in adjacent tissues. P, paracancerous tissue; T, tumor; Prx I, peroxiredoxin I; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Adenanthin induces apoptosis in pancreatic cancer cells

The above results suggest that adenanthin can significantly inhibit the proliferation and growth of pancreatic cancer

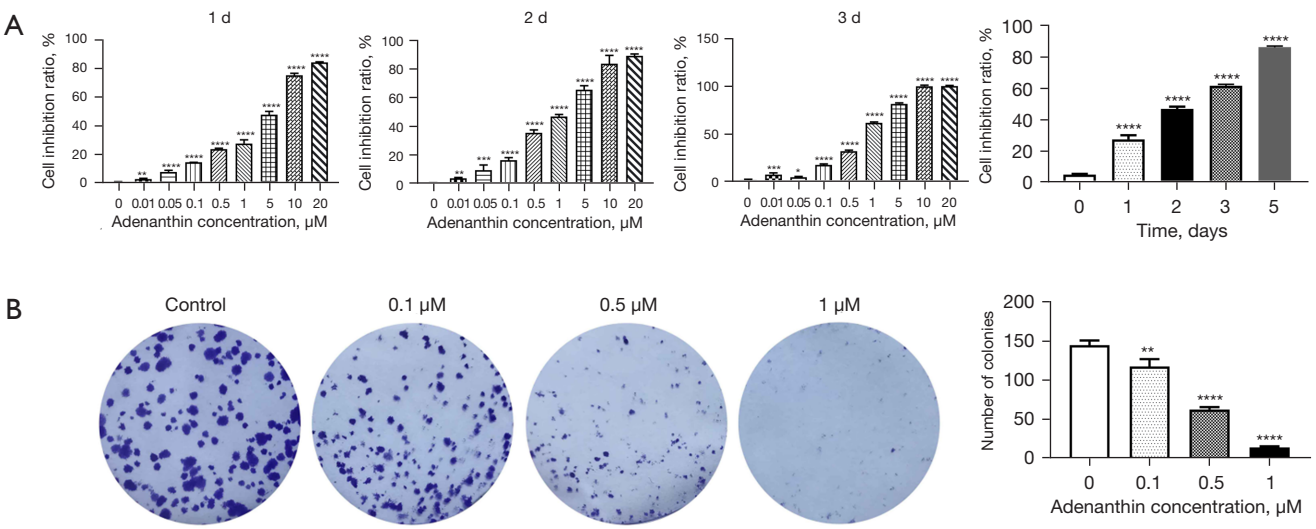

Figure 2 Adenanthin inhibits Aspc-1 cells proliferation. (A) The cell inhibition rate is time-dependent and concentration dependent. (B) Formation diagram of colonies with different concentrations; stained with 0.5% crystal violet (200× magnification). Compared with control group, *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001.

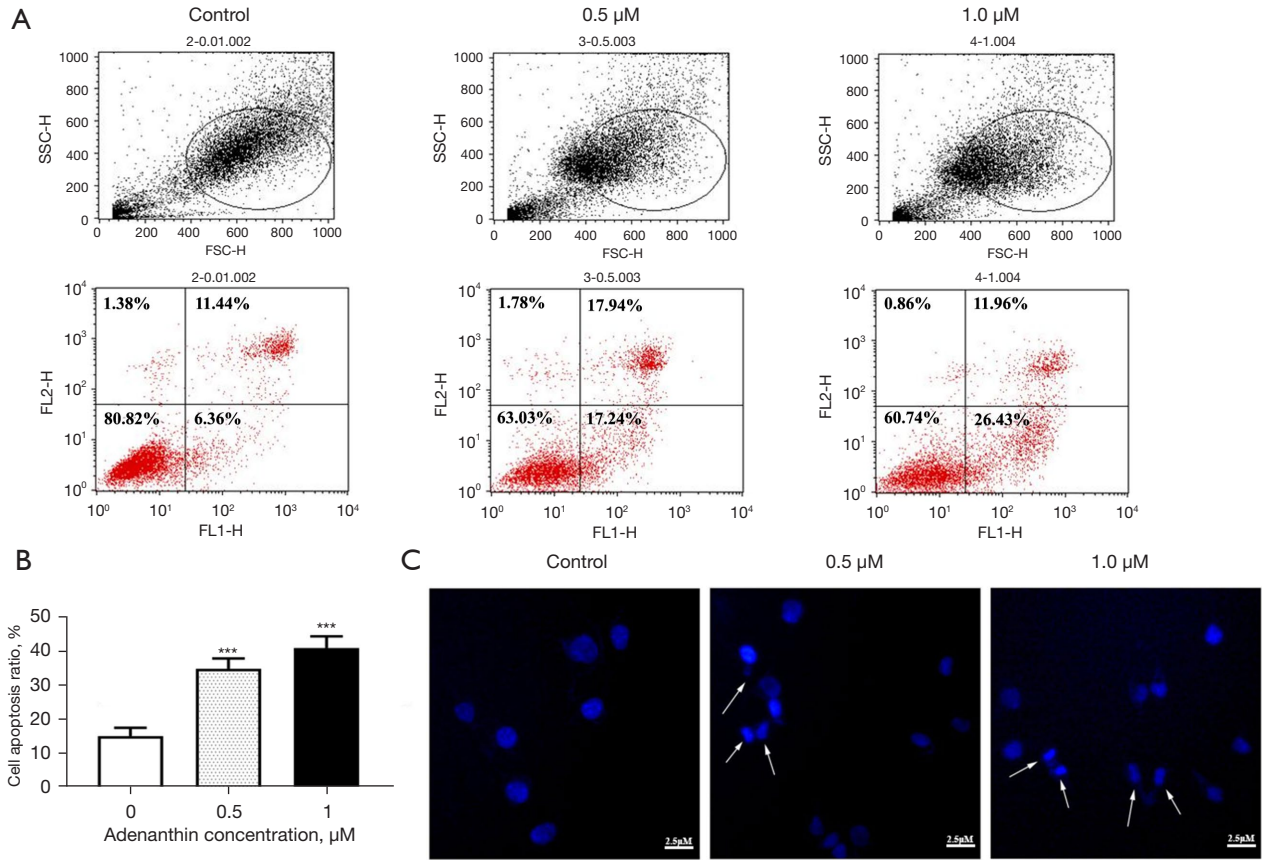

Figure 3 Adenanthin induces Aspc-1 apoptosis. (A) Detection of cell apoptosis rate by flow cytometry. (B) Statistical charts of apoptosis rates at different concentrations; compared with control group, ***, P<0.001, compared with control group. (C) Laser confocal uptake image after Hoechst 33258 staining (400× magnification), the arrows refer to apoptotic cells. SSC-H, Side Scatter-Height; FSC-H, Forward Scatter-Height; FL1-H, fluorescence channel 1-high; FL2-H, fluorescence channel 2-high.

cells. To investigate whether this inhibitory effect is associated with apoptosis, we used flow cytometry to detect apoptosis in cells after treatment with different concentrations of the drug for 48 hours. The results showed that treatment with adenanthin for 48 hours significantly induced apoptosis in Aspc-1 cells, with early apoptotic cell rates of 6.36%, 17.24%, and 26.43% for the dimethyl sulfoxide (DMSO)/0.5 μM/1 μM treatment groups, respectively (*Figure 3A*). The overall apoptosis statistics are shown in *Figure 3B*.

In the Hoechst 33258 staining experiment, after 48 hours of treatment with adenanthin, Aspc-1 cells exhibited nuclear condensation, chromatin aggregation, and the presence of apoptotic bodies. Moreover, with increasing concentrations, the number of cells decreased, the number of apoptotic bodies increased, a large number of cells exhibited cytoplasmic shrinkage, chromatin condensation, and fragmented nuclei, displaying typical apoptotic morphology (*Figure 3C*).

Adenanthin blocks the cell cycle in pancreatic cancer cells

Adenanthin can inhibit the proliferation and growth of cells by inducing apoptosis. To investigate whether adenanthin has an impact on the cell cycle, cells were incubated with different concentrations of adenanthin (1, 0.5, 0 μM) for 48 hours, followed by flow cytometry to assess the distribution of the cell cycle in pancreatic cancer cells after treatment with different concentrations of adenanthin. The results revealed that adenanthin significantly blocked Aspc-1 cells at the S phase and G2/M phase. The ratios of G1/S/G2M phases for the DMSO group were 60.21%/16.1%/17.38%, for the 0.5 μM compound treated for 48 hours were 2.49%/14.72%/74.92%,

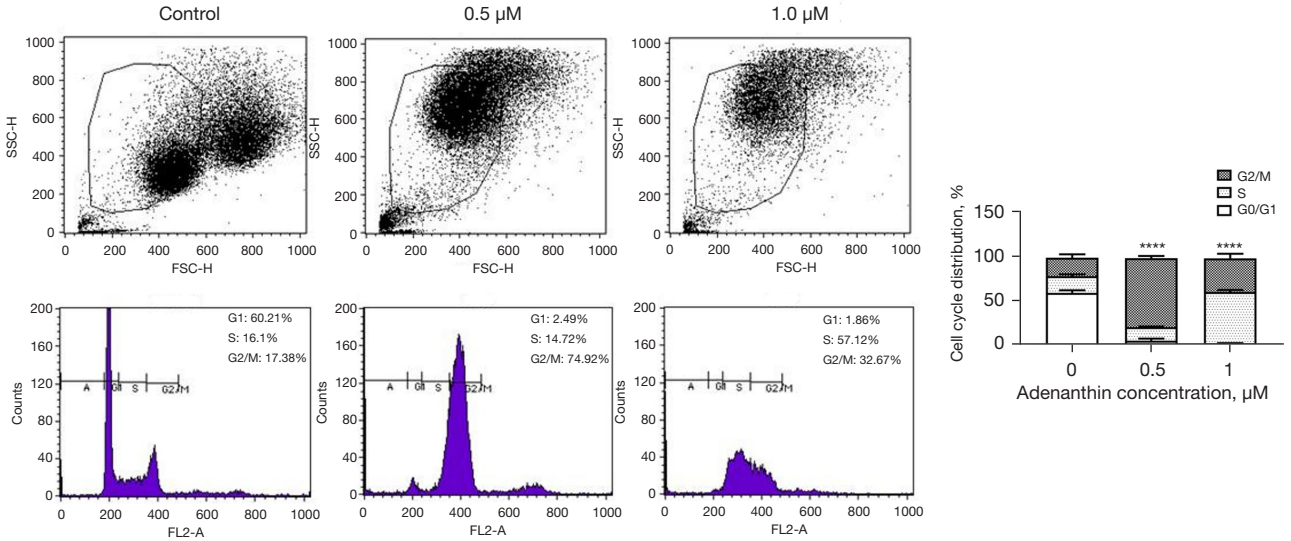

Figure 4 S and G2/M phases arrest induced by adenanthin in Aspc-1 cells. Results are expressed as mean ± SD. Statistical significance is indicated by ****, P<0.0001 *vs.* control group. SSC-H, Side Scatter-Height; FSC-H, Forward Scatter-Height; FL2-A, fluorescence channel 2-area; SD, standard deviation.

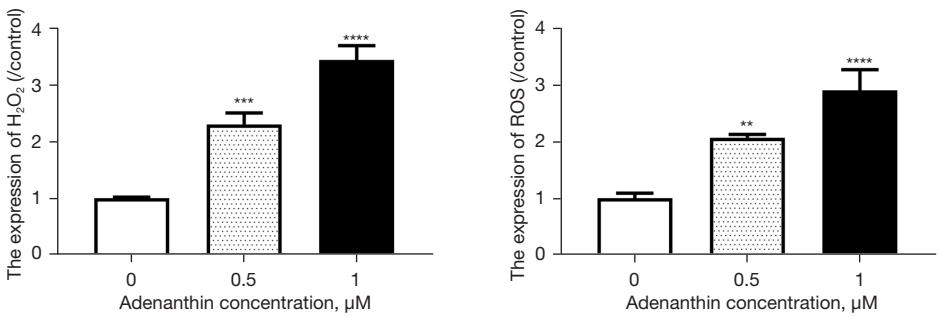

Figure 5 Adenanthin treatment significantly increased the content of H_2O_2 and ROS in pancreatic cancer cells. Compared with control group, **, P<0.01; ***, P<0.001; ****, P<0.0001. H_2O_2, hydrogen peroxide; ROS, reactive oxygen species.

and for the 1 μM compound treated for 48 hours were 1.86%/57.12%/32.67% (*Figure 4*). These results indicate that adenanthin can induce cell death and inhibit the growth of tumor cells by blocking the cell cycle.

Adenanthin increases H2O2 and ROS levels in pancreatic cancer cells

H_2O_2 is a common intermediate product of cellular oxygen metabolism in aerobic organisms. Current research suggests that tumor cells are more sensitive to H_2O_2 compared to normal cells, and elevated levels of H_2O_2 can lead to cell cycle arrest and apoptosis in tumor cells. Prx is the most important protein for degrading H_2O_2, and its role in tumors is also crucial. As a natural inhibitor of Prx, adenanthin may have a significant impact on the proliferation and apoptosis of pancreatic cancer by regulating H_2O_2. We measured the H_2O_2 concentration in Aspc-1 cells using an H_2O_2 detection kit and detected ROS levels in Aspc-1 cells using a ROS detection kit. The results showed that adenanthin treatment significantly increased the levels of H_2O_2 and ROS in pancreatic cancer cells (*Figure 5*).

Adenanthin affects the expression of apoptosis and cell cycle-related proteins

The aforementioned studies suggest that adenanthin can effectively inhibit the growth and proliferation of

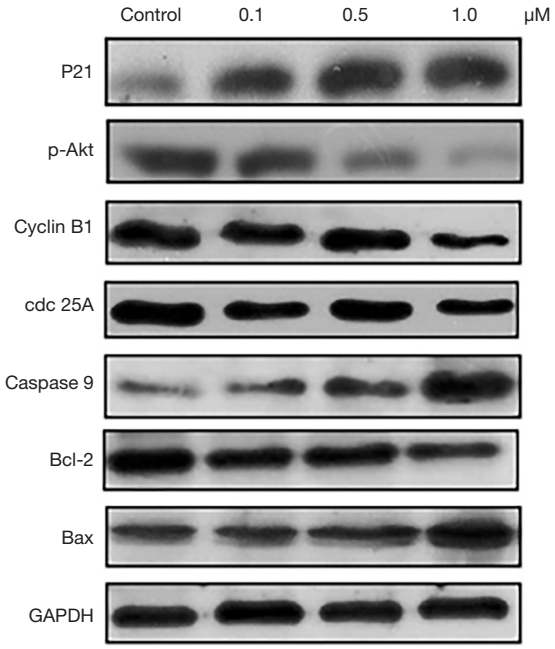

Figure 6 Cells were treated with adenanthin for 48 h and WB analysis were performed. GAPDH was used as a loading control. P21, cyclin-dependent kinase inhibitor 1; p-Akt, phosphorylated protein kinase B; cdc 25A, cell division cycle 25 homolog A; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WB, Western blot.

pancreatic cancer cells and can influence the proliferation and apoptosis of pancreatic cancer by regulating the levels of H_2O_2 and ROS. To further explore the underlying mechanisms of adenanthin's inhibitory effects on pancreatic cancer cells, we examined the expression of apoptosis and cell cycle-related proteins. The results showed that after 48 hours of adenanthin treatment, the expression of apoptosis-related proteins caspase 9 and Bax increased, while the anti-apoptotic protein Bcl-2 decreased in the cells. Cyclin B1, P21, and cdc 25A are all cell cycle-related proteins. When cells experience G2/M phase arrest, the expression of cyclin B1 and cdc 25A decreases, while P21 expression increases. Western blot analysis revealed an increase in P21 expression and a decrease in the expression of cyclin B1 and cdc 25A (*Figure 6*). These results indicate that adenanthin's inhibition of Prx I can lead to the regulation of H_2O_2 levels, exceeding the tolerance limit of pancreatic cancer cells, thereby causing DNA damage and promoting cell cycle arrest and apoptosis, ultimately leading to the death of pancreatic cancer cells.

In vivo validation of adenanthin's inhibitory effect on pancreatic cancer

To explore the inhibitory effect of adenanthin on pancreatic cancer *in vivo*, we established a subcutaneous xenograft model in nude mice. One group was given physiological saline as a control, another group was administered a low dose of adenanthin (10 mg/kg/day), and a third group was given a high dose of adenanthin (30 mg/kg/day). After euthanasia, the tumors were excised to measure their weight, and Western blot analysis was conducted to examine the expression of relevant proteins. The results showed that consistent with the *in vitro* studies, high-dose adenanthin treatment effectively controlled tumor growth. The expression of apoptosis-related proteins caspase 9 and Bax increased, while the anti-apoptotic protein Bcl-2 decreased. The expression of cell cycle proteins cyclin B1 and cdc 25A decreased (*Figure 7*).

Discussion

This study primarily investigated the role and regulatory mechanisms of adenanthin in the proliferation of pancreatic cancer cells. The findings revealed that adenanthin could significantly induce apoptosis and G2/M phase arrest in pancreatic cancer cells. To further explore its molecular mechanisms, we studied the expression of apoptosis proteins Bax/Bcl-2, caspase 9, and G2/M phase-related proteins cyclin B1, P21, and cdc 25A. We found that adenanthin-treated pancreatic cancer cells had increased expression of apoptosis proteins Bax/Bcl-2 and caspase 9, decreased expression of cell cycle-related proteins cyclin B1 and cdc 25A, and increased expression of P21. ELISA experiments further confirmed that adenanthin could significantly increase the levels of H_2O_2 and ROS, indicating that adenanthin can increase H_2O_2 levels to reach the tolerance limit of pancreatic cancer cells, thereby triggering DNA damage responses (DDR) and mitochondrial damage, causing cell cycle arrest in pancreatic cancer cells and inducing apoptosis, ultimately contributing to the treatment of pancreatic cancer. *In vivo* experiments in mice further confirmed the inhibitory effect of adenanthin on pancreatic cancer. Therefore, adenanthin may become a new drug for the treatment of pancreatic cancer.

Multiple studies have confirmed that adenanthin can specifically target Prx I/II and inhibit its activity, thereby exerting antitumor effects (4,5). Prx is a class of peroxidase

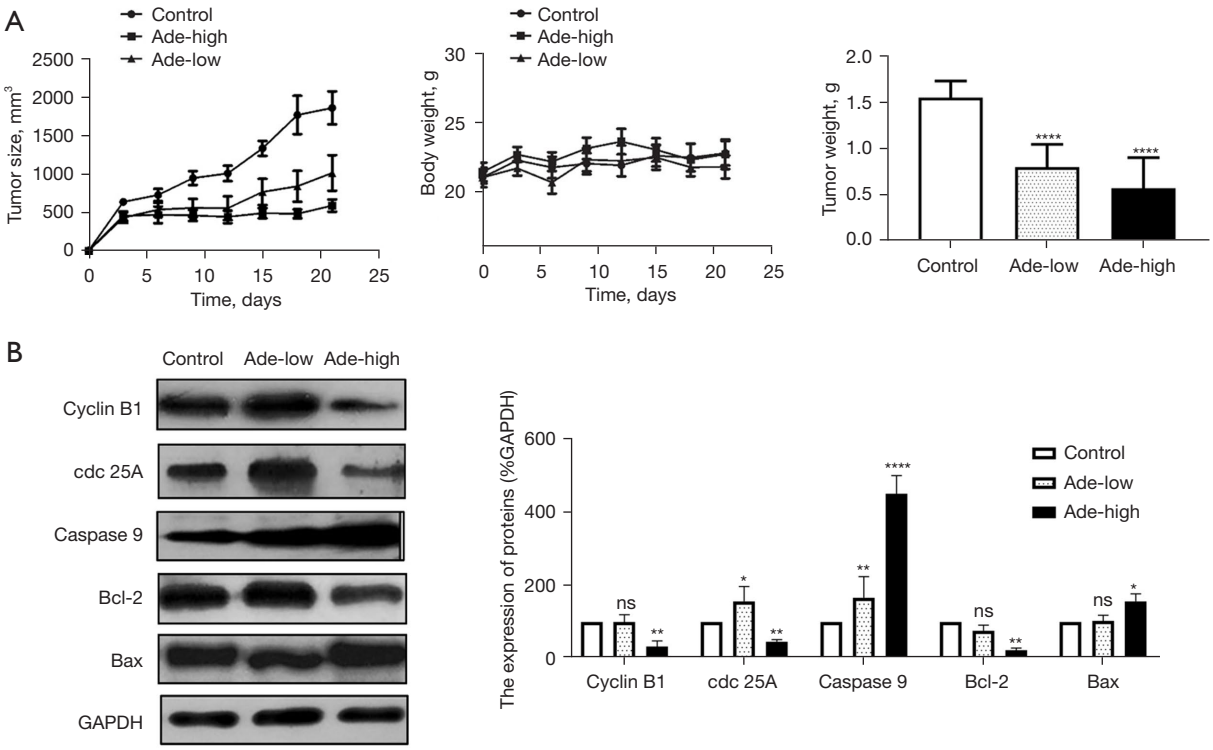

Figure 7 Subcutaneous transplantation tumor model was used to detect tumor inhibition rate and related protein expression. (A) After Ade-high treatment, the tumor was significantly controlled. (B) Nude mice were treated with adenanthin and WB analysis was performed. GAPDH was used as a loading control, *, P<0.05; **, P<0.01; ****, P<0.0001; ns, not significant. Ade-high, high-dose adenanthin group; Ade-low, low-dose adenanthin group; cdc 25A, cell division cycle 25 homolog A; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; WB, Western blot.

belonging to the superfamily of antioxidant proteins. Prx I is primarily distributed in the cytoplasm and nucleus and is involved in cell signaling, particularly in the regulation of tumorigenesis. It has been confirmed that Prx I is overexpressed in various tumors, including pancreatic cancer, breast cancer, colorectal cancer, etc., and its high expression is associated with the progression of pancreatic cancer (13). Our study found that the expression of Prx I in pancreatic cancer tissues was significantly higher than that in adjacent tissues, suggesting that it can be used as an auxiliary indicator for exploring pancreatic cancer prognosis. However, its regulatory role in tumors has not been fully elucidated. Reports have found that Prx I can promote the metastasis of pancreatic cancer through the regulation of the P38 pathway (14), but there is little reporting on its effect on pancreatic cancer proliferation. Adenanthin, as a natural inhibitor of Prx I, exerts an inhibitory effect on the proliferation of pancreatic cancer cells by inhibiting the activity of Prx I.

Study (15) has found that adenanthin can influence the development of obesity by regulating ROS and H_2O_2. Additionally, adenanthin can selectively kill hepatocellular carcinoma cells by inhibiting the activity of Prx I/II and causing ROS accumulation in cells (4). H_2O_2 is a common intermediate product of cellular oxygen metabolism in aerobic organisms (16). Tumor cells are more sensitive to H_2O_2 than normal cells and are more likely to die due to excessive or insufficient H_2O_2 levels. Low concentrations of H_2O_2 act as signaling molecules to regulate cell division and growth, but when the amount of H_2O_2 exceeds a certain critical value, the cell cycle can be arrested or apoptosis triggered (17). It is currently believed that excessive H_2O_2 causes DNA damage, thereby triggering complex DDR, ultimately leading to cell cycle arrest or apoptosis. DDR causes ataxia telangiectasia mutated (ATM) and Chk2 phosphorylation to increase, leading to the upregulation of P53 and P21, thereby causing cell cycle arrest (18-20); on the other hand, excessive H_2O_2 causes mitochondrial

damage, thereby initiating apoptosis, and H_2O_2 can also activate Caspase 3, thereby initiating apoptosis (21-24). We have reason to believe that the antitumor effect of adenanthin is through the regulation of H_2O_2 levels, thereby regulating ROS and increasing oxidative stress to promote tumor cell apoptosis and inhibit their proliferation. Our study found that adenanthin treatment significantly increased the levels of H_2O_2, ROS, and P21 expression in pancreatic cancer cells, and the changes in apoptosis and cell cycle proteins further confirmed this.

However, there are a few limitations of this study: Our study mainly preliminarily studied the inhibitory effect of adenanthin on pancreatic cancer cells through cell and mouse experiments. The research on the specific mechanism is still insufficient. Besides H_2O_2/ROS pathway, it is not clear whether there are other pathways. It is also unclear whether adenanthin inhibits the proliferation of pancreatic cancer through Prx I and clarify the role of Prx I. In the future, we will knock-out Prx I and study the exact mechanism by which H_2O_2/ROS regulates pancreatic cancer cell death.

Conclusions

Prx I is highly expressed in pancreatic cancer, and adenanthin, a small molecule inhibitor of Prx I, can exceed the tolerance limit of pancreatic cancer cells by regulating the levels of H_2O_2, thereby causing DNA damage and prompting pancreatic cancer cells to undergo cycle arrest and apoptosis, ultimately leading to the death of pancreatic cancer cells.

Acknowledgments

None.

Footnote

Reporting Checklist: The authors have completed the MDAR and ARRIVE reporting checklists. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-874/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-874/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-874/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of Suzhou Kowloon Hospital (approval number: Kowloon Ethics Review KY-2022-001), and informed consent was obtained from all the patients. Animal experiments were approved by Animal Experimentation Ethics Committee at Genepharma Company (No. IACUC-2022539), in compliance with institutional guidelines for the care and use of animals.

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