Radiation Protection of Polydatin Against Radon Exposure Injury of Epithelial Cells and Mice

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Abstract

Radon exposure is significantly associated with lung cancer. Radon concentration is currently reduced mainly by physical methods, but there is a lack of protective drugs or biochemical reagents for radon damage. This study aimed to explore the protective effect of polydatin (PD) on the radon-exposed injury. The results showed that PD can significantly reduce ROS level, raise SOD activity, weaken the migration ability, increase E-cad, and decrease mesenchymal cell surface markers (FN1, Vimentin, N-cad, α-SMA, and Snail) in radon-exposed epithelial cells. In vivo, PD increased the mice weight, promoted SOD activity, and decreased MDA content, the number of bullae, pulmonary septum thickness, lung collagenous fibers, and mesenchymal cell surface markers. Furthermore, PD inhibited p-PI3K, p-AKT, and p-mTOR expression. Compared with directly adding PD on radon-exposed cells, adding PD before and after radon exposure could more obviously improve the adhesion of radon-exposed cells, significantly alleviate the migration ability, and more significantly reduce mesenchyme markers and p-AKT and p-mTOR. These results indicate that PD can reduce oxidative stress, weaken epithelial-mesenchymal transition (EMT) and lung fibrosis in radon-exposed cells/mice, and have good radiation protection against radon injury. The mechanism is related to the inhibition of the PI3K/AKT/mTOR pathway.

Keywords

radon exposure, polydatin, epithelial-mesenchymal transition, radiation protection

Introduction

Radon, the only radioactive gas in nature, is the main form of background radiation.[1,2] It comes from the breakdown of uranium in soil and rock and is invisible, odorless, and tasteless. The α particles produced from radon and radon daughters can directly or indirectly damage DNA. For example, the α particles produced from radon can produce oxygen radicals to indirectly damage DNA, and can also directly damage DNA by DNA double-strand break, thus causing damage to exposed tissues.[3-5] A large number of studies have confirmed that radon is related to the occurrence of lung cancer. The higher the radon concentration, the greater the possibility of lung cancer. For example, in Ireland, those living in areas above the national reference level (200 Bq/m³) were 2.9–3.1 times more likely to be diagnosed with lung cancer than those living below the reference level.[6-8] Liu et al.

found that BEAS-2B cells showed malignant transformation after 20 generations of radon exposure (20 000 Bq/m³, 20 min/time, 3 d/time), with the increased ability of the soft

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agar-based clones and invasion by the soft agar colony formation assay, accompanied with mitochondrial structural and functional damage and abnormal energy metabolism.[9] Wei Ye successfully performed the tumor-forming experiment in nude mice using epithelial cells Rn30-40 contaminated with radon 30 times and passed 40 times.[10] In addition, epithelial-mesenchymal transition (EMT), accelerated cell proliferation, and decreased adhesion in epithelial cells occur at the early stage of radon exposure (Rn6).[11,12]

With the research on the radon exposure damages in the world and the improvement of people's attention to health, the standards and specifications on radiation safety at home and abroad put forward relevant requirements for monitoring and limiting radon to reduce the health threat of radon exposure. For example, the U.S. Environmental Protection Agency[13] and the National Radiation Protection Committee[5] recommended that indoor radon concentration should be controlled at 150 Bq/m^3 and 200 Bq/m^3, respectively. According to the latest data, WHO recommended that the reference level of indoor radon concentration should be 100 Bq/m^3. Under some specific conditions, such as high background radiation areas, the reference level should not exceed 300 Bq/m^3.[14] However, the radon concentration in some places is still very high, such as high background areas and mines, and even the radon concentration in some mines can reach hundreds of thousands of Bq/m^3.[15,16] Residents or workers living in these areas still have the risk of radon exposure. Radiation protection against radon exposure damage has always been a major problem. The radon hazards are prevented mainly by reducing radon concentration through physical methods, such as site selection during building construction, building materials selection, mechanical ventilation, improvement of mining methods, and reduction of radon exhalation. Furthermore, there is still a lack of drugs or preparations for preventing radon radiation damage.

Studies have shown that radon exposure can induce oxidative stress, inflammatory response, and EMT to increase the malignant degree of epithelial cells, leading to lung injury.[12] Most of the reported radon injury protection drugs are realized by antioxidation, such as melatonin[17] and compound Chinese medicine preparation composed of Cordyceps sinensis, Astragalus membranaceus, and Salvia miltiorrhiza.[18] Based on the previous research of our group, polydatin (PD), a polyphenol-rich phytoalexin, has many biological characteristics such as remarkable antioxidation, anti-inflammation, anti-tumor, and immunomodulation,[19] with little toxic and side effects and high safety.[20,21] Studies have found that PD can regulate cell proliferation, promote cell apoptosis, and reduce oxidative stress and inflammatory response through signaling molecules such as P53 and SIRT1.[22-25] It protects against ionizing radiation injury and ischemia-reperfusion injury of tissues and organs caused by oxidative stress and inflammatory reaction. It has been proved that PD can effectively alleviate acute inflammation and pulmonary fibrosis and attenuate lung injury caused by γ-ray.[26] Polydatin can also

protect against X-ray-induced intestinal injury by increasing the proliferation of crypt epithelial cells and microvessel density and lessening intestinal endothelial cell apoptosis.[27] Whether PD also has the protective effect on radon exposure injury has not been reported so far.

Therefore, in this study, PD radiation-protected cell and mouse models were constructed through an ecological radon chamber to determine the radiation protection of PD against radon-exposed injury and the possible mechanism of the protection of PD.

Materials and methods

Cell culture and cell model construction

Human bronchial epithelial cells (BEAS-2B and 16HBE) were purchased from Shanghai Zhongqiao Xinzhou Biological Co., Ltd, and cultured in a complete medium supplemented with growth factors and .1% penicillin-streptomycin. The growth factors included essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, and trace minerals, but no serum was contained. Cells were cultured in an incubator at 37°C and 5% CO_2.

Two types of BEAS-2B and 16HBE cells in good condition were inoculated on a transwell chamber, which was placed in a radon exposure device. The cells in the chamber were directly exposed to the 20 000 Bq/m^3 radon and its progeny from a radium source for 20 minutes, and this procedure was repeated after 3 days. The cells exposed twice were used as the first generation exposed-radon cells (Rn1), and the Rn6 cells were selected to carry out follow-up experiments. There were 2 ways to administer PD in the cell model: one was to directly add 100 μmol (Rn6 + PD100) or 200 μmol (Rn6 + PD200) PD to the radon-exposed cell for 24 hours, the other was to add 200 μmol PD (PDRn6) before and after each radon exposure of epithelial cells. The control cells were placed in a natural background radon concentration device.

Cell adhesion assays

Cell adhesion was defined by the cell adhesion kit of BestBio. In brief, the 96-well plate was coated with 100 μL/well coating solution overnight at 4°C for the first day, and the plate was washed with washing solution 3 times. The cells were seeded with 5×10^4 cells/well (5 wells for each group) and cultured at 37°C for 1 h. After being washed with the culture medium 2–3 times, 100 μL culture medium was added in each well, and then cells were incubated with 10 μL staining solution B for 1 hours at 37°C. The absorbance A450 value was detected by a microplate Reader (Biotek Synergy2, USA).

Cell migration assays and scratch assays

The cells were collected and inoculated into the upper chamber (8 μm pore size) at 2.5×10^5 cells/well in the growth

factor-free basic medium. The culture medium containing growth factors was added into the lower chamber and cultured for 8 h (16HBE cells) and 20 h (BEAS-2B cells) in a 5% CO_2, 37°C. The cells were fixed with methanol for 30 minutes and stained with .1% crystal violet for 20 minutes. Then, non-migrating cells in the chamber were wiped off with a cotton swab, gently washed with PBS, and imaged under an inverted light microscope (Olympus, Japan) with three wells for each group and three times repeat.

Scratch test: the 6-well plate was inoculated with appropriate epithelial cells for the next day covered. After 24 hours in a 5% CO_2, 37°C culture, the cells were scratched with a sterile ruler and pipette, washed with PBS three times, and added to a medium containing 20% growth factor. The cells were placed in a 5% CO_2, 37°C culture and photographed at 0 hours, 24 hours, and 36 hours. The area of scratch blank area in the picture was measured by ImageJ software. The cell mobility was calculated by the ratio of the blank area at other time points to the blank area at 0 hours.

Immunofluorescence staining

The cells in different groups were inoculated on slides with 2×10^5 cells cultured for 24 hours. The next day, cells were rinsed with PBS three times and fixed in 4% paraformaldehyde for 15 minutes at room temperature. After being washed with PBS three times again, the cells were penetrated with .5% Triton X-100 for 15 minutes, sealed with the goat serum for 30 mintes at room temperature, and incubated with antibodies of E-cad (1: 200) and Vimentin (1:500) overnight at 4°C. After being washed with PBS, cells were incubated in the second antibody for 1 hours at room temperature, and the nucleus was re-stained with DAPI for 10 minutes and washed with PBS three times. The slide was sealed with an anti-fluorescence quencher and observed under a fluorescence microscope (Leica, Germany).

Animal model construction

Male mice (6 weeks, 18 ± 2 g) from Changzhou KawenSi Experimental Animal Center (NO.201928357) were randomly divided into 4 groups: control group (Con), radon exposure group (120 Working Level Month (WLM)), radon exposure + PD50 (120 WLM + PD50), and radon exposure + PD100 (120 WLM + PD100) (n = 6 of each group).

One week after adaptive feeding, the mice were placed in an HD-3 multifunctional radon chamber to inhale radon gas for building a radon exposure experimental animal model. Mice in PD groups were intraperitoneally injected with 200 μL at 50 and 100 mg/kg for 30 minutes before each radon exposure. The 120 WLM group was given an equal amount of normal saline. The mice inhaled 10^5 Bq/m^3 radon gas in a multifunctional ecological radon chamber for 10 hours every day, 6 days every week, and reached 755 hours, equivalent to the cumulative exposure dose of radon 120 WLM. Mice could

eat and move freely during radon exposure. Control mice with normal diets were raised in the background radiation-level environment. The experimental protocol was approved by the Ethics Committee of Soochow University.

Histopathology staining

After radon exposure, blood and lung tissues were collected from mice of each group within 24 hours. The blood was placed on ice for 30 minutes, then centrifuged at 3000 r for 10 minutes at 4°C, and serum was separated and kept at −80°C. Lung tissues from each group were divided into two parts. One part was put in dry ice and then kept in a −80°C freezer as a spare. The part in good form was fixed with 4% paraformaldehyde at room temperature, dehydrated by ethanol gradient, embedded in paraffin, sliced, dehydrated, and hydrated. The histopathology of lung tissue stained with HE and deposition of collagen fibers in lung tissue by Masson's trichrome (Meilunbio, G1345) staining was observed under a microscope.

Oxidative stress detection

According to the instructions of ROS, MDA, and SOD detection kit of Nanjing Jiancheng Institute of Bioengineering, the MDA content and SOD activity in the serum of mice were detected. The ROS and SOD activity of radon-exposed epithelial cells were detected.

Immunohistochemistry

Paraffin sections were routinely dewaxed and rehydrated. After high-pressure antigen repair, they were placed in a 3% catalase solution for 10 minutes to remove endogenous peroxidase. After being washed with PBS 5 minutes × 3 times, the sections were sealed with goat serum for 30 minutes at room temperature. The sections were incubated with first antibodies α-SMA (1:200, CST), Vimentin (1:800, Abcam), FN1 (1: 500, Abcam), and Snail (1:300, CST) overnight at 4°C. The next day, sections were washed with PBS and incubated with a secondary antibody for 30 minutes at room temperature. Subsequently, tissues were washed with PBS, showed with DAB solution, re-dyed in hematoxylin, dehydrated with gradient ethanol, and imaged.

Western blot

The protein of the cell clusters or lung tissue was extracted with RIPA lysate containing protease inhibitor. After quantification by the BCA method, SDS-PAGE gel electrophoresis was carried out with 80 V, 30 minutes and 120 V, 90 minutes, respectively. The protein was transferred from the gel to the PVDF membrane at 300 mA, 2 hours. The membrane was sealed with 5% skim milk powder at room temperature and incubated overnight at 4°C with primary antibodies

(E-cad (1: 3000, Abcam, USA), Vimentin (1: 5000, Abcam), FN1 (1: 5000, Abcam), Snail (1: 1000, CST, USA), α-SMA (1: 1000, CST), N-cad (1:1000, CST), p-PI3K (1: 500, CST), p-AKT (1: 1000, CST), p-mTOR (1: 800, CST), and GAPDH/β-tubulin). The PVDF membrane was washed with TBST three times and incubated with the rabbit/mouse secondary antibody for 60 minutes at room temperature. Signals were detected with the ECL kit. The quantitative analysis was carried out by Image J software.

Statistical analysis

All data were expressed as mean ± S.D. Statistical analysis was performed by analysis of variance or LSD-t test using SPSS23.0 or GraphPad Prism software. $P < .05$ is considered statistically significant. All experiments were repeated at least three times.

Results

Polydatin reduced oxidative stress level of radon-exposed cells

The ROS level and SOD activity of radon-exposed epithelial cells were detected to explore the effect of PD on the oxidative stress of radon-exposed cells. The results showed that the SOD activity in Rn + PD100, Rn + PD200, or PDRn6 groups was higher than that in the Rn6 group (Figures 1(a) and 1(b)). On the contrary, the ROS level in PD groups decreased (Figures 1(c)–1(e)), and the ROS level of the PDRn6 group was the lowest among all groups, indicating that PD alleviated the oxidative damage of radon-exposure cells.

The SOD activity of radon-exposed 16HBE (A) and BEAS-2B cells (B) was detected. (C) The ROS levels of

radon-exposed 16HBE and BEAS-2B cells were analyzed. (D, E) The fluorescence intensity was quantified by ImageJ software. * *: Compared with Rn6, $P < .01$; #: compared with Rn6 + PD100, $P < .05$; and: compared with Rn6 + PD200, $P < .05$.

Polydatin improved cell adhesion and reduced the migration ability of radon-exposed cells

The adhesion of each group was tested to determine the effect of PD on the adhesion of radon-exposed cells. For 16HBE-Rn6 cells, 100 and 200 μmol PD could improve the cell adhesion, but there was no statistical difference ($P > .05$, Figure 2(a)). In BEAS-2B cells, only 100 μmol PD increased the adhesion ability (Figure 2(b)). However, the cell adhesion of PDRn6 in 16HBE and BEAS-2B cells was significantly higher than Rn6 ($P < .01$, Figures 2(a) and 2(b)). The results suggest that adding PD before and after radon exposure has a better protective effect on radon exposure injury.

Transwell migration and scratch tests were used to determine the effect of PD on the migration ability of radon-exposed epithelial cells. Scratch test results showed that compared with Rn6 cells, the wound healing of radon-exposed cells slowed down with PD, and the healing speed of 200 μmol PD was slower than that of 100 μmol. PDRn6 cells had the largest wound area and the slowest healing speed (Figures 2(c)–2(f)). Transwell migration test showed that PD reduced the migration number of radon-exposed cells in the upper chamber compared with alone radon-exposed ones, and the migration number of the PDRn6 group was the lowest among all groups of 16HBE and BEAS-2B (Figures 2(g)–2(i)). The results indicate that PD can weaken the migration ability of radon-exposed cells, and the trend of weakening is more obvious in the high-concentration PD group.
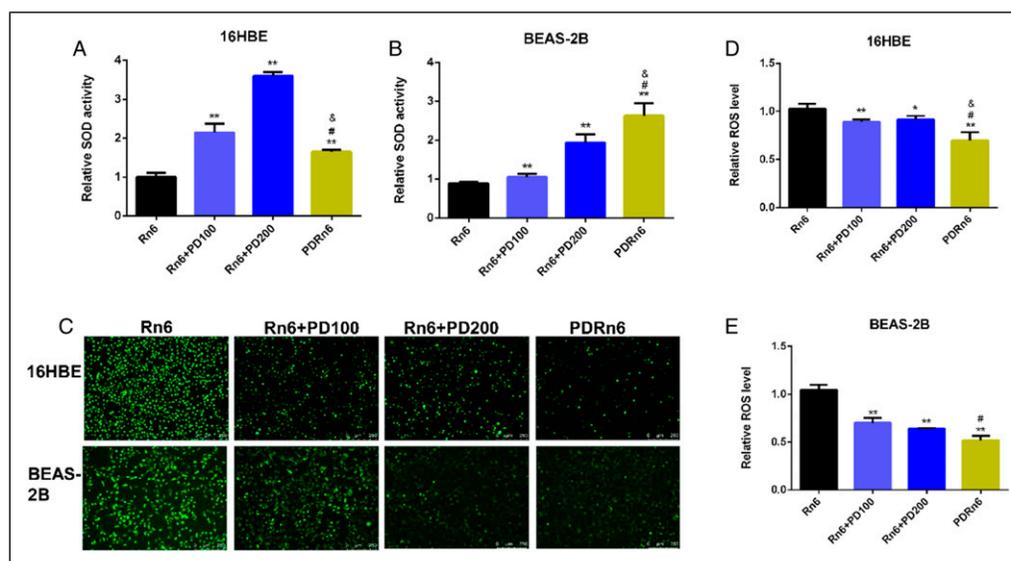

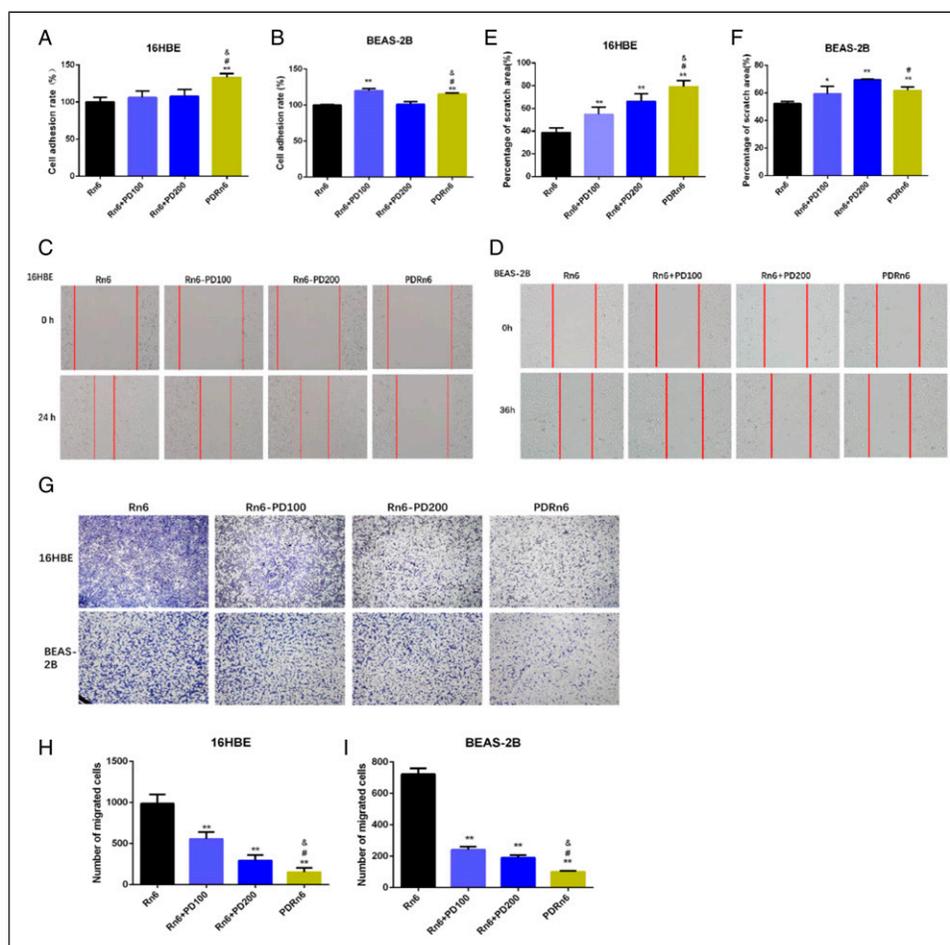**Figure 1.** PD can reduce the oxidative stress level of radon-exposed cells.

Figure 2. PD can reduce cell migration ability and improve cell adhesion. (A, B) The adhesion kit was used to detect the adhesion ability of radon-exposed 16HBE and BEAS-2B epithelium cells. (C, D) The migration ability of the cells in each group was analyzed by Scratch test. (E, F) Quantitative analysis of the scratch area in different groups was drawn with Image J software. (G) The migration ability of radon-exposed 16HBE and BEAS-2B cells was detected by the transwell migration test. (H, I) The number of migrating cells was quantified by ImageJ software. * *: Compared with Rn6, $P < .01$; #: compared with Rn6 + PD100, $P < .05$; and: compared with Rn6 + PD200, $P < .05$.

Polydatin increased E-cad and inhibited Vimentin protein in radon-exposed cells

Radon exposure can induce EMT in bronchial epithelial cells.[12] The EMT markers were detected by immunofluorescence to understand the effect of PD on the EMT of radon-exposed cells. The E-cad protein and Vimentin protein, important markers in EMT, were detected. Compared with the alone radon-exposed group, 100 and 200 μmol PD decreased the Vimentin protein in radon-exposed 16HBE and BEAS-2B cells. Furthermore, PD increased the expression of E-cad in epithelial cells with radon exposure. In the PDRn6 group, the E-cad protein was the highest, and the Vimentin protein was the lowest (Figure 3).

An immunofluorescence test was used to detect the expression of E-cad and Vimentin protein in radon-exposed cells of 16HBE (A) and BEAS-2B (B). (C) The fluorescence intensity was quantified by ImageJ software. * *: Compared with Rn6, $P < .01$; #: compared with Rn6 + PD100, $P < .05$; and: compared with Rn6 + PD200, $P < .05$.

For further study of the protective effect of PD on radon-exposed cells, immunoblot analysis was applied to detect EMT markers in radon-exposed 16HBE and BEAS-2B cells with PD. The results showed that directly adding 100 and 200 μmol PD increased the expression of E-cad and decreased the interstitial cell markers of N-cad, FN1, Vimentin, and α-SMA in radon-exposed cells. The expression of transcription factor Snail protein in radon-exposed cells was also decreased by PD (Figures 4(a) and 4(b)). In addition, adding PD before and after radon exposure increased E-cad more significantly and reduced N-cad, FN1, Vimentin, α-SMA, and Snail more effectively than other groups (Figures 4(c) and 4(d)). These results suggest that PD can attenuate the EMT of epithelial cells induced by radon radiation.
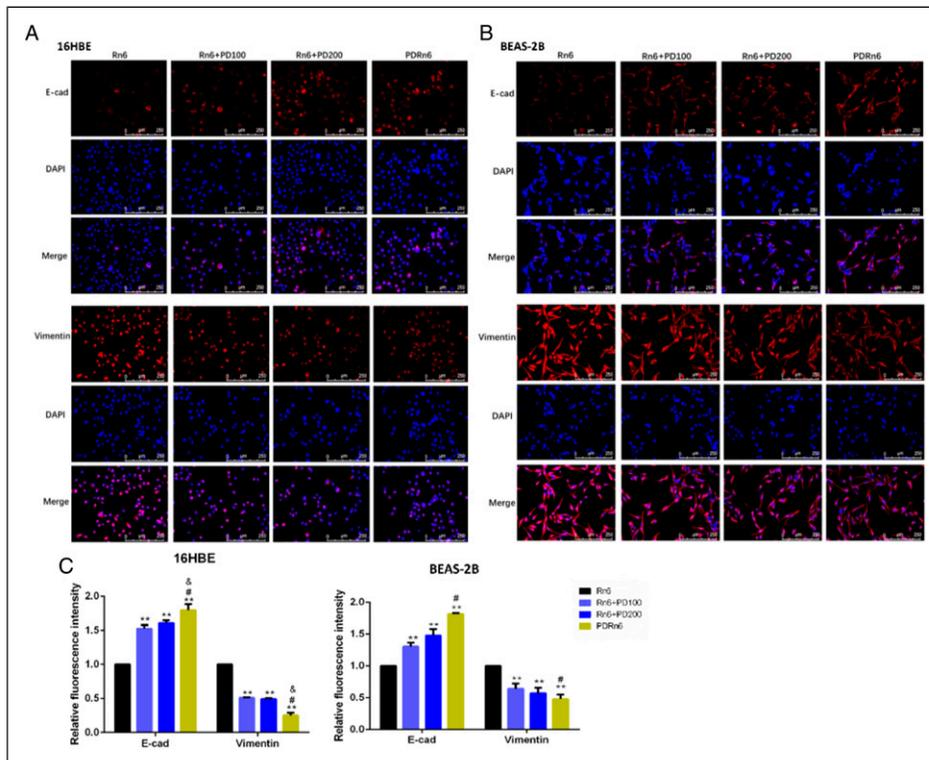
Figure 3. PD can increase E-cad and decrease Vimentin in radon-exposed cells.

Polydatin protected the radon-exposed damage in mice

In vivo, the weight of mice was not significantly different in each group at 60 WLM in this study. When the cumulative dose of radon exposure reached 120 WLM, compared with the alone radon exposure group, 50 mg/kg PD alleviated the weight loss of mice, while the weight of mice in the 100 mg/kg PD group had no statistical change (Figure 5(a)). Compared with 120 WLM, the serum SOD activity in 50 and 100 mg/kg PD groups was increased, and the SOD activity in 100 mg/kg group was the highest (Figure 5(b)). In addition, compared with 120 WLM, the serum MDA content in PD group decreased, but the serum MDA in 120 WLM + PD50 was the lowest (Figure 5(c)). The results indicate that PD can reduce the oxidative stress in radon-exposed mice.

HE staining results showed that, compared with 120 WLM, the thickening of the pulmonary septum, inflammatory reaction, and inflammatory hydrophilic cells in the PD group were reduced, the number of pulmonary bullae was also obviously reduced, the thickening of the pulmonary septum was relieved, and the improvement was more obvious in high PD group (Figure 5(e)). Masson staining results showed that compared with 120 WLM, the deposition of collagen fibers in lung tissue of mice with PD decreased, and the deposition of collagen fibers in lung tissue of mice in 100 mg/kg group was the least (Figure 5(d)). The results suggest that PD can reduce lung injury and collagen fiber deposition in mice induced by

high-dose radon exposure, and the effect of high-concentration PD is better.

The immunohistochemical test indicated PD lessened the expression of EMT proteins in lung tissue of radon-exposed mice with 50 or 100 mg/kg compared with 120 WLM. In addition, the expression of FN1, α-SMA, Vimentin, and Snail in lung tissue decreased with PD, and the higher PD, the more obvious the decreasing trend (Figures 5(f) and 5(g)). The results indicate that PD can protect the EMT in the lung induced by radon exposure.

Polydatin inhibited the PI3K/AKT/mTOR pathway in radon-exposed cells

Radon exposure can induce EMT in epithelial cells by activating the PI3K/AKT/mTOR pathway.[12] PI3K/AKT/mTOR protein expression was detected by western blotting to further explore how PD exerts the radioprotective effect of radon exposure. Compared with Rn6, PD significantly reduced the expression of p-AKT and p-mTOR (Figures 6(a)–6(c)). In vivo, the expression levels of p-PI3K, p-AKT, and p-mTOR protein in the lung tissue in the PD group were lower than those in the 120 WLM group (Figures 6(d) and 6(e)). The results were consistent with those in vitro. Therefore, PI3K/AKT/mTOR pathway can be involved in the radiation protection of PD against radon exposure injury. In addition, compared with Rn6 + PD100 or Rn6 + PD200, the p-mTOR protein in the PDRn6 group decreased, and compared with the
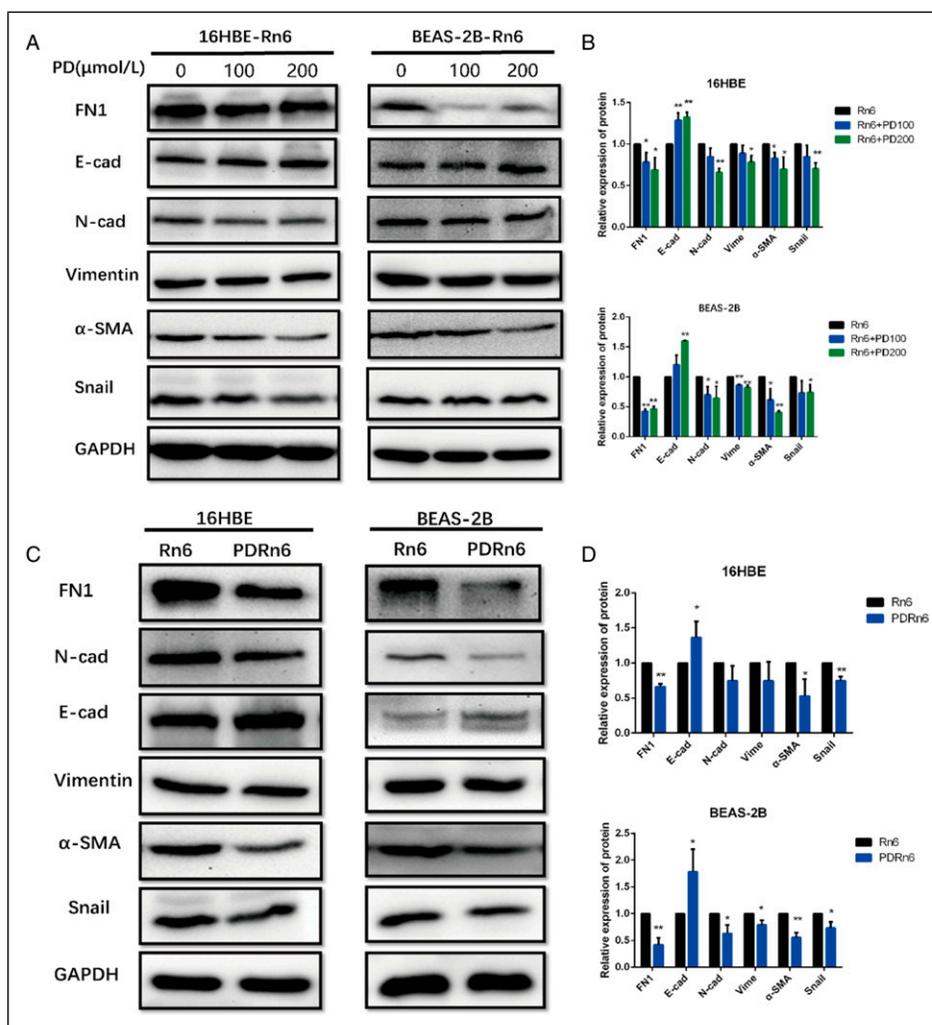
Figure 4. PD can regulate the expression of EMT markers in radon-exposed cells. (A) The protein expression of E-cad, Vimentin, N-cad, FN1, α-SMA, and Snail in radon exposure model of 16HBE and BEAS-2B cells with directly adding PD. (C) The protein expression of E-cad, Vimentin, N-cad, FN1, α-SMA, and Snail in radon-exposed 16HBE and BEAS-2B cells with PD added before and after radon exposure. (B, D) The expression level of EMT markers in different groups was quantified by Image J software. *: Compared with Rn6, $P < .05$; **: Compared with Rn6, $P < .01$.

100 μmol PD group, p-AKT in PDRn6 was also significantly down-regulated (Figure 6(c)). The results suggest that using PD before and after radon exposure for many times has better radiation protection against radon exposure injury.

Discussion

WHO and many countries have enacted a series of limits to reduce the health hazards of radon exposure. The radon concentration is mainly reduced by physical methods to prevent radon hazards, such as site selection during building construction, building materials selection, mechanical ventilation, improvement of mining methods, and reduction of radon exhalation. However, radon is still high in high background areas and some special places, and even some of the mine radon concentration can be as high

as hundreds of thousands Bq/m^3. For example, the old uranium radon concentration in Poland is more than 28 000 Bq/m^3, and the uranium radon concentration in Utah is 1.85×10^5 Bq/m^3. Therefore, it is necessary to develop radiation protection drugs. Polydatin has been used to prevent and treat many diseases because of its remarkable anti-inflammatory and antioxidant abilities.[28,29] Studies showed that PD has a protective effect in different ways. For example, in acute severe hemorrhagic shock, PD can protect hepatocytes by activating the Sirt1 receptor, increasing SOD activity, and weakening lysosome and mitochondrial damage caused by oxidative stress.[30] In radiation protection, PD can improve cell vitality or reduce oxidative stress levels by reducing the damage to cell morphology and ultrastructure induced by radiation. Polydatin can also improve the efficiency of homologous
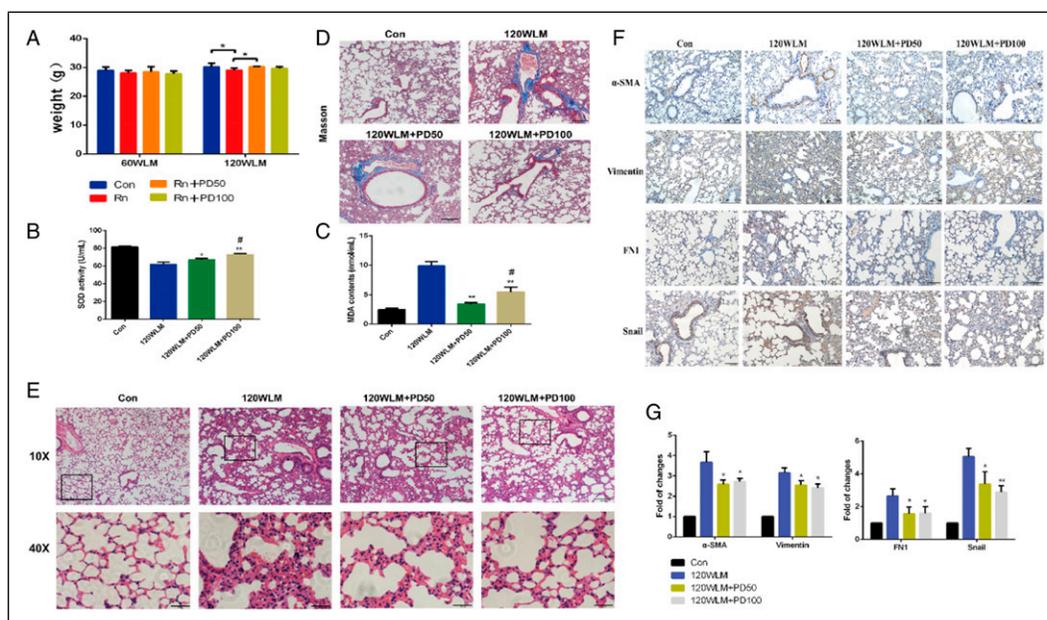
Figure 5. PD can reduce lung tissue injury in radon-exposed mice. (A) The weight changes of mice in each group. (B) Serum SOD activity of mice in each group. (C) MDA content in the serum of all groups. (D) Changes of pulmonary fibrosis in each group. (E) Pathological changes in lung tissue in each group. (F) Expression of EMT-related proteins in the lung tissue of each group was detected by immunohistochemistry. (G) The expression level of EMT markers was quantified with Image J software. *: Compared with 120 WLM, $P < .05$; **: Compared with 120 WLM, $P < .01$. #: Compared with 120 WLM + PD50, $P < .05$.
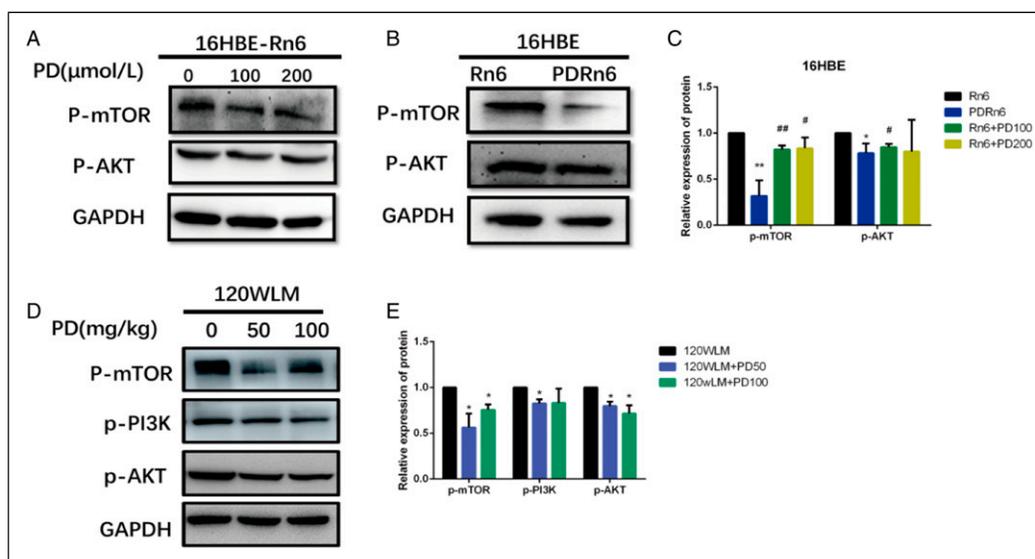

Figure 6. PD can inhibit the PI3K/AKT/mTOR pathway activation induced by radon exposure. (A) The expression level of p-AKT and p-mTOR after adding PD directly to the radon exposure model of 16HBE cells. (B) p-AKT and p-mTOR protein expression levels of radon-exposed 16HBE cells in each group with PD added before and after radon exposure. (D) The effect of PD on the protein expression levels of p-PI3K, p-AKT, and p-mTOR in lung tissue of radon-exposed mice. (C, E) The expression levels of proteins were quantified with Image J software. *: Compared with Rn6/120 WLM, $P < .05$; **: Compared with Rn6, $P < .01$; #: compared with PDRn6, $P < .05$.

recombination and repair and reduce DNA damage and apoptosis in radiation injury.[31-33]

Our previous studies found that radon exposure could raise cell migration ability, reduce cell adhesion, regulate EMT markers, induce EMT in epithelial cells, and subsequently cause radiation damage.[12] Based on the research foundation of another subject in our group,[34] the radiation protection of PD against radon damage was explored in this paper. The results

show that PD can reduce ROS levels, increase SOD activity, and protect cells from radon exposure hazards. In addition, PD can improve the expression of E-cad protein, reduce the migration ability, and weaken the damage of radon-exposed cells. These results are consistent with previous results. It is reported that PD can reduce HIF-1a protein expression, weaken the activation of the Hedgehog signaling pathway, and reduce the ability of cell proliferation, migration, and invasion, thus inhibiting EMT of gastric cancer cells under hypoxia.[35] In addition, the results in this study show that PD can alleviate the inflammatory reaction of lung tissue, thickening of the pulmonary septum, and formation of pulmonary bullae in radon-exposed mice, and reduce the deposition of collagen fibers and the expression of α-SMA in lung tissue, thus alleviating the lung tissue injury and pulmonary fibrosis induced by radon exposure. Yan Hao et al[36] found that PD could weaken the alveolar inflammatory reaction induced by radiation, reduce the content of MDA in lung tissue, increase the activities of SOD and Gpx1 enzymes and their gene expression, and alleviate the inflammatory reaction of lung tissue to play a role in radiation protection. Polydatin can also scavenge free radicals, relieve the degree of pulmonary hemorrhage and edema, lower Vimentin and α-SMA protein expression, regulate the imbalance of Th1/Th2, and effectively remit acute lung inflammation and advanced pulmonary fibrosis induced by radiation via inhibiting the TGF-β-Smad3 pathway and EMT, thus alleviating radiation-induced lung injury.[26]

Furthermore, the present study found that 0–300 μmol PD weakened the protein expression level of epithelial-mesenchymal cell surface markers induced by radon exposure, and the higher the PD concentration, the more obvious the weakening trend (Supplementary Figure 1). At mRNA level, adding 200 μmol PD before and after radon exposure down-regulated FN1 and α-SMA genes and up-regulated E-cad gene in 16HBE-Rn6 cells, but had no effect on BEAS-2B-Rn6 cells (supplementary Figure 2). At the protein level, whether adding PD directly or before and after radon exposure, the protein expression level of FN1, α-SMA, and Snail in radon-exposed cells decreased, the protein expression level of E-cad increased, and the EMT in epithelial cells induced by radon exposure was reduced. Furthermore, PD reduced the expression level of p-AKT and p-mTOR in radon-exposed cells. In vivo, PD also reduced the protein expression level of p-PI3K, p-AKT, and p-mTOR, inhibited the progress of EMT and fibrosis in lung tissue of radon-exposed mice, and weakened lung injury induced by radon exposure. The results suggest that the protective effect of PD on radon-exposed injury is related to the PI3K/AKT/mTOR pathway. Recent studies have found that PI3K/Akt/mTOR signaling pathway plays an important role in the proliferation, apoptosis, and metastasis of malignant cells and the antagonism of radiotherapy and chemotherapy. PI3K-AKT-mTOR signal pathway regulates the synthesis of ribosomes and protein and angiogenesis, thus promoting the infinite proliferation of cells.[37]

Previous studies have found that PD increases E-cad via AKT/GSK-3β/Snail pathway, decreases N-cad and Snail, and reverses EMT, thus inhibiting the migration and invasion of colon cancer cells.[38] Compared with directly adding PD to radon-exposed cells, adding PD before and after each radon exposure can increase cell adhesion, reduce the number of cell migrations, and decrease p-mTOR and p-AKT. The results indicate that adding PD before and after each radon exposure improves radiation protection against radon exposure damage. The results are consistent with the findings of Yahyapour,[39] which has found that giving PD for a long time before and after irradiation can more effectively alleviate pneumonia and pulmonary fibrosis induced by high-dose radiation.

The latest data showed that 100 μM PD could significantly reduce the apoptosis of GC-1 cells induced by 2 Gy X-ray and relieve the damage of germline cells induced by ionizing radiation via regulating the expression of Tnp2 and Ppp1c and, reducing ROS and cell apoptosis.[40] In addition, PD can inhibit the proliferation and apoptosis of macrophages induced by acetaminophen, improve the morphology and physiological process of macrophages, and enhance the body's immunity.[41] Polydatin has been shown to inhibit MERS-CoV infection in vitro and prolong cell survival after viral infection. Polydatin inhibits the expression of MERS-CoV nucleocapsid protein, which is essential for viral replication, and MERS-CoV-induced host cell apoptosis, indicating that PD may also be effective in treating SARS-CoV-2 infection.[42] Thus, PD of plant-based origin has been proposed for prophylactic/combination treatment of individuals at risk of COVID-19, such as asymptomatic or asymptomatic contacts.[43] In conclusion, PD alleviates oxidative stress, improves body immunity, and protects against radiation injury and lung injury, suggesting that it has good application value.

This study shows that PD can reduce the migration ability, improve cell adhesion, increase E-cad protein, and weaken the interstitial cell markers, such as Vimentin, FN1, N-cad, α-SMA, and Snail, thus weakening the EMT induced by radon exposure in epithelial cells. Additionally, adding PD before and after exposure has a better protective effect. Polydatin can relieve lung tissue injury and fibrosis in radon-exposed mice by antioxidation and has good radiation protection against radon radiation injury. The protective effect of PD on radon injury may be related to its inhibition of the PI3K/AKT/mTOR signaling pathway. In this study, the protective effect of PD on radon injury is confirmed, and the related mechanism is discussed, which provides a reference for the protection from radon exposure injury. However, this study also has certain limitations. No sequencing analysis was performed to identify differential targets in lung tissue with or without PD intervention for radon exposure. Instead, according to the previous research results of the research group, indicators such as EMT and PI3K/AKT/mTOR signaling pathways were directly detected. In addition, the effects of different dosing modalities (e.g., oral administration and intraperitoneal injection) on the prevention of radon exposure

injury were not verified, and only one intraperitoneal injection was used. We will consider further refinement of the study in the future.

Appendix

Abbreviations

BEAS-2B	Human bronchial epithelial cell strain
16HBE	Human bronchial epithelial cell strain
EMT	Epithelial-mesenchymal transition
FN1	Fibronectin 1
PD	Polydatin
Rn	Radon
GSK-3β	Glycogen synthase kinase 3β
E-cad	E-cadherin
N-cad	N-cadherin
α-SMA	α-smooth muscle actin

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