PDTC inhibits apoptosis and phenotypic transformation of co-culture of myeloma cells and renal tubular epithelial cells by reducing the secretion of light chain protein

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Abstract

Background The human myeloma cell line RPMI-8226 is co-cultured with human proximal tubular epithelial cell HK-2, which is commonly used to simulate the renal environment in patients with multiple myeloma. This study aimed to investigate the effects of NFκB inhibitor PDTC on the viability, apoptosis and cell phenotype of HK-2 cells in the co-culture system of myeloma cells in renal tubular epithelial cells.

Methods An in vivo environment was simulated through a cell co-culture system. RPMI-8226 cells and HK-2 cells were inoculated in the co-culture chamber and cultured for 24 h to establish the co-culture system. PDTC was used in the single culture group and the co-culture group, respectively. The activity of HK-2 cells and RPMI-8226 cells in each group was detected by MTT. An immunoturbidimetric assay was performed to assess the effect of PDTC on secretion of κ light chain and λ light chain in RPMI-8226 cells. Flow cytometry was used to detect apoptosis of HK-2 cells. Western blot was carried out to detect NF-κB activation in RPMI-8226 myeloma cells, as well as the expression levels of caspase-3, bcl-2, Bax, E-cadherin, and α-SMA in HK-2 cells. Caspase-3 assay kit was used to detect the activity of caspase-3. The effect of PDTC on the secretion of κ light chain and λ light chain of RPMI-8226 cells was detected by immunoturbidimetry and the ratio was calculated.

Results PDTC had a potent inhibitory effect on proliferation of RPMI-8226 cells in a dose- and time-dependent manner. PDTC had no significant effect on the viability of HK-2 cells cultured alone, and the addition of PDTC to the co-culture system significantly increased the viability of HK-2 cells. PDTC did not significantly change the apoptosis of HK-2 cells cultured alone, but could reduce the apoptosis of renal tubular epithelial cells by regulating the activity of caspase3 and the ratio of bcl2/bax in HK-2 cells in the co-culture system. After PDTC treatment, the expression of cell surface marker E-cadherin decreased, and the expression of α-SMA increased, which induced the renal interstitial fibrosis. The secretion of κ light chain and λ light chain of RPMI-8226 cells was significantly decreased after the addition of PDTC, but the ratio was not changed.

Conclusions PDTC can inhibit the cell activity, promote apoptosis, and reduce the secretion of κ light chain and λ light chain through inhibiting the NF-κB pathway activation of myeloma cell RPMI-8226, leading to increased activity of renal tubular epithelial cells HK-2 in the co-culture system, decreased apoptosis, and renal interstitial fibrosis.

1. Introduction

Multiple myeloma (MM) is a hematological malignancy that often occurs in the elderly. It is characterized by abnormal proliferation of monoclonal plasma cells. Clinical manifestations vary according to the degree of tumor infiltration, often involving multiple organs. Patients with MM whose first symptom is associated with kidney damage often ignore their conditions due to insidious onset. About 20%-40% patients with MM had different degrees of kidney damage when they were first diagnosed. In normal physiology, light chains are synthesized in plasma cells faster than heavy chains. As a result, there are
always extra light chains in the system during the process of immunoglobulin synthesis. The unused light chain proteins are then broken down by renal tubules. This is a normal process and does not cause any harm to the kidney. When MM occurs, however, syntheses of both light and heavy chains are accelerated, resulting in a large amount of light chain proteins remaining in the blood system. The proximal renal tubules’ scavenging ability to remove unused light chain proteins is thus overwhelmed, forcing extra light chain proteins to enter into the distal tubules and form a large number of casts. Cast formation in the renal tubules increases the tubular pressure, and reduces the glomerular filtration rate, making it even more difficult to scavenge the extra light chain proteins. While the concentration of light chain proteins keeps increasing, a vicious circle is formed, leading to and further aggravating kidney damage.

According to a study, the cell viability of HK-2 cells treated with different MM cell lines was significantly decreased, and the apoptosis was also significantly increased. However, it was found by sequencing that the secretory vesicles of the MM cell line contain renal pathogenic miRNAs, and whether the apoptosis of HK-2 cells is related to the light chain protein secreted by the MM cell line has not been elucidated. It was reported that the light chain obtained in patients with end stage renal disease can enter HK-2 cells to produce sufficient amount of hydrogen peroxide to stimulate the production of monocyte chemoattractant protein-1 (MCP-1), while MCP-1 is a key chemokine for proximal tubule activation. Therefore, this study aimed to investigate whether the light chain protein secreted by myeloma cells in the co-culture system affects the activity and apoptosis of renal tubular epithelial cells HK-2.

In addition to symptomatic supportive therapy and renal replacement therapy, anti-myeloma medications such as thalidomide, lenalidomide and bortezomib are often used in the treatment of myeloma nephropathy. Although these medications are of great help in the treatment of MM, they are less effective and have many side effects and drug resistance in some refractory MM because they have no targets with specificity, leading to MM chemotherapy failure and recurrence. Therefore, it is of great significance to develop some new drugs that can specifically act on the target for the treatment and prevention of drug resistance and recurrence of MM. According to published studies, increased activity of NF-κB was observed in myeloma cell lines and fresh bone marrow mononuclear cells from patients with myeloma, compared to normal bone marrow mononuclear cells, which may be related to the mutations of NF-κB, genetic polymorphisms, intercellular contact, and receptors capable of activating NF-κB which only exist in malignant plasma cells. NF-κB can play an anti-apoptotic role by up-regulating interleukin-1, interleukin-2, macrophage colony-stimulating factor and other anti-apoptotic factors. It has been found that α-terpineol can inhibit the proliferation of MM cell line RPMI-8226 by inhibiting NFkB. In view of the important role of NFκB in myeloma, the treatment of NFκB targets has become a hot spot for the treatment of MM. In a previous study, renal tubular epithelial cells reabsorb the light chain can activate the NFκB pathway in the cell, producing a series of chemokines such as MCP-1 to promote the activation of proximal tubular epithelial cells, but PDTC can inhibit light chain-induced production of MCP-1; however, this previous study only explored the effect of light chain in late kidney patients on NFκB pathway in HK-2 cells, and did not clarify whether PDTC directly affects HK-2 cells cultured alone or HK-2...
cells in co-culture systems. Therefore, this study set up multiple experimental groups to investigate the effects of PDTC on myeloma cells RPMI-8226 and human HK-2 cells in the co-culture system.

In summary, the human myeloma cell line RPMI-8226 was co-cultured with human proximal tubular epithelial cells HK-2 to simulate the renal environment in patients with multiple myeloma in this study. This study explored the effects of PDTC, a specific inhibitor of NFκB, on a series of biological functions of myeloma cells and renal tubular epithelial cells in a co-culture system in order to provide a reference to clinical treatment of MM nephropathy by targeting NF-κB.

2. Materials And Methods

2.1. Materials and reagents

The materials and reagents used in this study were purchased from commercial sources: human proximal tubular epithelial cell line HK-2 and human myeloma cell line RPMI-8226 were purchased from ATCC cell bank; fetal bovine serum (FBS), DMEM cell culture medium, and PRIM1640 cell culture medium were purchased from Gibco; NF-κB inhibitor PDTC was purchased from Selleck (catalog #: S3633); MTT cell proliferation and cytotoxicity assay kit (catalog #: C0009), caspase-3 activity assay kit (catalog #: C1115) and BCA kit (catalog #: P0009) were purchased from Beyotime Biotechnology Co., Ltd.; 6-well plate, 24-well plate and co-culture chamber (0.4 µm pore size) were purchased from BD; dimethyl sulfoxide (DMSO) was purchased from sigma; apoptosis assay kit was purchased from Sungene Biotech Co. Ltd. (catalog #: AO2001-02P-H); caspase-3 (catalog #: 9662), B cell lymphoma 2 (bcl-2, catalog #: 2872), B cell lymphoma 2 associated X protein (Bax, catalog #: 2774), E-cadherin (catalog #: 3195), α-smooth muscle actin (α-SMA, catalog #: 19245), and IκBα (catalog #: 4812) were purchased from CST; internal reference GAPDH (catalog #: 10494-1-AP) and horseradish peroxidase (HRP)-labeled secondary antibody (catalog #: SA00001-2) were purchased from Proteintech Group Inc. Immunoturbidimetric assay was performed on a Beckman Array 360 automatic protein analyzer (Beckman, USA). Consumable reagents such as diluents, standards, buffers, etc. were all purchased from Beckman. A 10 mM stock solution of DMSO was prepared.

2.2. Cell cultures

HK-2 and RPMI-8226 cells were cultured respectively in DMEM and PRIM1640 media supplemented with 10% FBS. The cell cultures were maintained at 37 °C in a humidified incubator supplied with 5% CO₂. The media were changed every 2 days. Cells were passaged when reaching 80% confluency.

2.3. Establishment and grouping of co-culture system of myeloma cells and renal tubular epithelial cells

A co-culture system was established by seeding RPMI-8226 cells at 1 × 10⁵ cells per well in the upper chamber of a 6-well transwell and seeding HK-2 cells at 3 × 10⁵ cells per well in the lower chamber. The cells were divided into HK-2 alone culture group, HK-2 alone + PDTC culture group, RPMI-8226/HK-2 co-
culture group, and RPMI-8226/HK-2 + PDTC co-culture group after cultured for 24 h. In the HK-2 alone culture group and HK-2 alone + PDTC culture group, HK-2 cells were seeded in the lower chamber, whereas in the upper chamber were added RPIM-1640 medium only for the HK-2 alone culture group and RPIM-1640 medium containing 25 µM PDTC for the HK-2 alone + PDTC culture group. In the RPMI-8226/HK-2 co-culture group and RPMI-8226/HK-2 + PDTC co-culture group, HK-2 cells were seeded at $3 \times 10^5$ cells per well in the lower chamber, and in the upper chamber were seeded RPMI-8226 cells at $1 \times 10^5$ cells per well for both groups. In addition, a supplement of 25 µM PDTC was added into the upper chamber for the RPMI-8226/HK-2 + PDTC co-culture group.

2.4. MTT assay

Log phase RPMI-8226 cells were harvested by digestion with 0.25% trypsin. The cells were re-suspended in the medium and diluted to $3 \times 10^4$ cells per mL. To a 96-well plate was added the cell suspension at 100 µL per well. After the cells were attached, RPIM-1640 media containing a nal concentration of 0, 10, 20, 30, 40, and 50 µM PDTC were added to sextuplicate wells. A blank well without cells was set for each concentration. The plate was incubated for 2 days, and cell viability was checked every 24 h. In the RPMI-8226/HK-2 co-culture group and RPMI-8226/HK-2 + PDTC co-culture group, RPMI-8226 cells were seeded at 5000 cells per well in the upper chamber of a 24-well plate. In the lower chamber of the 24-well plate, HK-2 cells were seeded at 5000 cells per well for the two co-culture groups as well as the two HK-2 alone culture groups. After the cells were attached, corresponding amount of the medium was added to the upper chamber for the HK-2 alone culture group and the RPMI-8226/HK-2 co-culture group. For the HK-2 alone + PDTC culture group and RPMI-8226/HK-2 + PDTC co-culture group, DMEM medium containing a nal concentration of 25 µM PDTC was added to the upper chamber. Viability of the HK-2 cells in the lower chamber was measured after incubation for 24 h. The assay protocol was described below. MTT solution (5 mg/mL) was added to the plate, followed by incubation at 37 °C for 4 h. Then the culture solution was carefully aspirated, and 150 µL of DMSO was added. After shaking in the dark at room temperature for 10 min, absorbance of each well was measured using a microplate reader at 450 nm. Cell viability was calculated using formula: cell viability = (experimental well OD value – blank well OD value) / (control well OD value – blank well OD value) × 100%. IC$_{50}$ was calculated with GraphPad Prism software and was used as the drug concentration in the co-culture systems.

2.5. Immunoturbidimetric assay for light chain proteins

Concentrations of κ light chain and λ light chain in the RPMI-8226/HK-2 co-culture group and RPMI-8226/HK-2 + PDTC co-culture group were measured by scatter immunoturbidimetric assay using a Beckman Array 360 protein analyzer in accordance with the manufacturer’s manual. The ratio of κ light chain to λ light chain κ/λ was calculated.

2.6. Effect of PDTC on apoptosis of renal tubular epithelial cells by flow cytometry
For all the 4 groups, after 24 hours of incubation, supernatant in the lower chamber was aspirated, and cells were digested with trypsin. The cells were rinsed with PBS, followed by centrifugation at 1200 rpm to remove the supernatant. According to the instructions of the apoptosis detection kit, 1 mL of 1 × binding buffer was added to the cells. The supernatant was aspirated after centrifugation at 1200 rpm. The cells were re-suspended in 1x binding buffer to get a concentration of 1 × 10^6 cells/mL. To 100 µL of the cell suspension in each group was added 5 µL of Annexin V-FITC. After mixing, the mixture was incubated at room temperature for 10 min in the dark, followed by adding 5 µL of PI solution. After mixing, the mixture was incubated at room temperature for 5 min in the dark. PBS was added to get a total volume of 500 µL. After mixing, the mixture was subjected to flow cytometry. Apoptotic rate was sum of the early apoptotic rate (lower right quadrant) and the late apoptotic rate (upper right quadrant).

2.7. Total cell protein extraction and western blotting

Cells were harvested after centrifugation at 3000 rpm following trypsic digestion. Appropriate amounts of RIPA lysate and protease inhibitor were added, followed by ultrasound on ice for 5 min. After complete lysis, the mixture was centrifuged at 12000 g for 15 min in a low temperature centrifuge. The supernatant was collected, and 10 µL was used for total protein concentration measurement using BCA assay. To the remaining supernatant was added 5 × loading buffer, followed by heating at 100 °C for 10 min. Equal amounts of total proteins for the 4 groups were loaded on gels containing 5% stacking gel and 10% separation gel for electrophoretic analysis. The gels were run at a constant voltage of 80 V until bromophenol blue entered the stacking gel with minimal distortion of the bands, when the voltage was changed to 120 V until the target bands were separated. The protein bands were transferred from gel to PVDF membrane by wet transfer method under a constant current of 275 mA for 80 min. After the membrane was blocked in TBST buffer supplemented with 5% milk at room temperature for 2 h, corresponding diluted primary antibody (dilution factor: 1:1000) was added. For cells in the upper chamber, levels of IκB protein were measured to assess the NF-κB pathway activation. For cells in the lower chamber, levels of caspase-3, bcl-2, Bax, E-cadherin, and α-SMA were measured. The membrane was incubated at 4 °C overnight within the corresponding primary antibody. After washing, the secondary antibody supplemented with 2% milk was added, followed by incubation at room temperature for 1 h. After development, the image was analyzed using Image J software for gray values of the bands. GAPDH was used as an internal reference. The ratio of the gray value of the target protein to the gray value of GAPDH was regarded as the expression level of that protein.

2.8. Caspase-3 Activity Assay

Caspase3 activity detection kit was used to detect Caspase-3. pNA standard curve was measured according to the instructions of the kit, and then 100 ul cell lysate was added to every 2 × 106 cells after treatment, and the supernatant was taken after ultrasonic lysis. 40 ul of buffer solution, 10 ul of Ac-DEVD-pNA (2 mM) and 50 ul of sample to be tested were added into the 96-well plate, and a blank control group was set to exclude the background absorption value. The mixture was incubated at 37°C for 2 h, and OD value of the sample was detected with a microplate analyzer at the wavelength of 405 nm. The OD value of the sample to be tested was determined after subtracting the OD value of the blank control group. The
standard curve was used to calculate how much pNA was catalyzed in the sample, which was expressed as multiple of the control group.

### 2.9. Statistical Analysis

The SPSS 20 software was used for statistical analysis. Data were expressed as mean ± standard deviation. The paired t test was used for comparison between groups. A difference was statistically significant when \( p < 0.05 \). Each test was repeated more than three times.

### 3. Results

#### 3.1. Effect of NF-κB inhibitor PDTC on the activity of HK-2 in co-culture system

As shown in Fig. 1a, PDTC had a potent inhibitory effect on proliferation of RPMI-8226 cells. Its inhibitory effect on cell proliferation increased with higher drug concentration and longer time of action, thus demonstrating a dose- and time-dependence. The half-inhibition concentrations (IC\(_{50}\)s) of PDTC calculated by software were 25.59 µM and 3.03 µM, respectively, for 24 h and 48 h. The concentration of 25 µM was thus chosen for subsequent experiments.

As shown in Fig. 1b, compared with HK-2 single culture, the activity of HK-2 cells in single culture was not significantly changed after the addition of PDTC (\( p > 0.05 \)), while the activity of HK-2 cells in co-culture system was significantly decreased after the co-culture with RPMI-8226 (\( p < 0.05 \)), but activity of HK-2 cells was significantly increased after the addition of PDTC in co-culture system.

#### 3.2 Effect of PDTC on apoptosis and cell phenotype transformation of HK-2 cells in co-culture system

The apoptosis rates of HK-2 cells in the single culture group, the single culture + PDTC group, the co-culture group, the co-culture + PDTC group were 0.20 ± 0.03, 0.27 ± 0.05, 4.93 ± 0.46, 2.12 ± 0.11, respectively, and there was no significant difference in the apoptosis rate of renal tubular epithelial cells between the single culture group and the single culture + PDTC group (\( p > 0.05 \)); compared with the single culture group, the apoptosis rates of HK-2 cells and RPMI-8226 cells in co-culture group were significantly increased, and the difference was statistically significant (\( p < 0.05 \)); compared with the co-culture group, the apoptosis rate of HK-2 cells in co-culture + PDTC group significantly decreased, and the difference was statistically significant (\( p < 0.05 \)). Figure 2

In addition, this study also explored the effect of PDTC on the apoptosis-related proteins caspase3, bcl2 and bax in HK-2 cells in the co-culture system to further elucidate the effect of PDTC on the apoptosis of renal tubular epithelial cells in MM disease state. The results are shown in Fig. 3. After adding PDTC, there was no significant difference in the activity of caspase3 and the ratio of bcl2 to bax in HK-2 cells in single culture (\( p > 0.05 \)). Compared with single HK-2 cell culture group, the activity of caspase3 in HK-2 cells in co-culture system was significantly increased, and the ratio of bcl2 to bax was significantly
decreased ($p < 0.05$). Compared with the co-culture group, the activity of caspase3 in HK-2 cells decreased significantly after the addition of PDTC in the co-culture system, and the ratio of bcl2 to bax was significantly increased ($p < 0.05$). Therefore, the above results indicate that PDTC reduced the apoptosis process of HK-2 cells in the co-culture system by reducing apoptosis-related proteins. In addition, by analyzing the HK-2 cell surface markers in the co-culture system (Fig. 3d and 3e), it was found that the E-cadherin on the surface of HK-2 cells decreased significantly after the PDTC treatment in the co-culture group, and α-SMA increased significantly ($p < 0.05$). The results showed that PDTC can induce morphological changes of renal epithelial cells and produce renal interstitial fibrosis.

3.3 Effect of PDTC on RPMI-8226NFκB pathway in myeloma cells

By Western blot analysis of RPMI-8226 cells in co-culture group and co-culture + PDTC group (Fig. 4), it was found that IκB protein levels were significantly increased after 24 h of 25 umol/L PDTC treatment, and the relative expression increased from $0.70 \pm 0.07$ to $0.99 \pm 0.02$, with statistically significant difference ($p < 0.05$). The above results indicate that PDTC can increase the IκB protein of myeloma cell RPMI-8226 in the co-culture system, thereby inhibiting the activation of NFκB pathway.

3.4 Effect of PDTC on secretion of light chain proteins in myeloma cells

As shown in Table 1, levels of both κ light chain and λ light chain were reduced in the presence of 25 µM PDTC for 24 h. The differences were $8.30 \pm 1.39$ and $0.91 \pm 0.35$, respectively, and both were statistically significant ($p < 0.05$). However, there was no significant difference in the ratio of κ light chain level to λ light chain level ($p > 0.05$).

| Group                           | κ light chain level | λ light chain level | κ/λ       |
|---------------------------------|--------------------|--------------------|-----------|
| RPMI-8226/HK-2 co-culture group | 16.45 ± 1.62       | 1.70 ± 0.23        | 9.85 ± 2.02 |
| RPMI-8226/HK-2 + PDTC co-culture group | 7.92 ± 1.04*       | 0.77 ± 0.15*       | 10.67 ± 2.72 |

* $p < 0.05$, compared with RPMI-8226/HK-2 co-culture group

4. Discussion

MM is a malignant tumor characterized by proliferation of plasma cells in bone marrow. It accounts for about 1%-2% of all cancers, and about 10% of hematological malignancies. In the United States, MM has become the second most common blood cancer just after non-Hodgkin's lymphoma\textsuperscript{17}. A Bence Jones
protein (BJP) is a free immunoglobulin light chain found in the blood. It can freely pass through the glomerular filtration membrane due to its small molecular weight. It was believed that kidney damage due to BJP was associated with BJP's catalytic activities, including catalyzed amide cleavage, catalyzed hydrolysis of peptide and catalyzed DNA cleavage\textsuperscript{18-20}. Therefore, it was postulated that inhibition of BJP’s catalytic activities would attenuate BJP-mediated apoptosis\textsuperscript{21}. Sanders et al\textsuperscript{22} reported that κ light chain protein can enter the nucleus and activate lysosomes, causing vacuoles and cell lysis, and eventually leading to apoptosis. Although there have been studies\textsuperscript{4} showing that the cell activity of HK-2 was significantly decreased and apoptosis was significantly increased after treated with medium cultured with different MM cell lines, but renal pathogenic miRNA was found in the secretory vesicles of MM cell lines by sequencing. So whether the apoptosis of HK-2 cells is related to light-chain protein secreted by MM cell line has not been clarified. In this study, HK-2 human proximal tubular epithelial cells were co-cultured with RPMI-8662 myeloma cells. It was found that the light chain proteins produced by myeloma cells can mediate apoptosis of HK-2 renal tubular epithelial cells. The underlying mechanism may be related to the catalytic activity of light chain proteins.

Light chain proteins in urine are not only catalytically active, but also directly toxic to renal tubular epithelial cells. Both in vitro and in vivo experiments showed that urinary proteins can cause damage to renal tubular epithelial cells, thereby aggravating renal interstitial lesions\textsuperscript{23}. Damaged tubular epithelial cells secrete large amounts of pro-fibrotic factors, including transforming growth factor-β1 (TGF-β1), which promote fibrosis formation\textsuperscript{24}. In this process, epithelial-mesenchymal transition (EMT) plays a very important role. Damaged tubular epithelial cells downregulate epithelial cell surface marker proteins, promote cell cycle arrest, secrete pro-fibrotic factors, and promote expression of key genes such as α-SMA and snail\textsuperscript{25,26}. In this study, it was found that the epithelial cell surface marker E-cadherin was significantly down-regulated, while the interstitial cell surface marker α-SMA was significantly upregulated when HK-2 cells were co-cultured with RPMI-8226 cells. This finding indicated that light chain proteins can cause damage to renal tubular epithelial cells, mediating cell phenotype transformation such as EMT.

NF-κB is an important nuclear transcription factor that plays a crucial role in various biological processes, including the body's inflammatory response, immune response, apoptosis/anti-apoptosis, and cell cycle regulation\textsuperscript{27}. Activation of NF-κB pathway was observed in myeloma cell lines and myelomonocytes of patients with myeloma\textsuperscript{10,15}. This observation was related to its anti-apoptotic effect. The NF-κB family is composed of five members, including RelA (p65), RelB, c-Rel, p50 and p52, which form various dimeric complexes. When cells are stimulated by external stimuli, IκB kinase is activated, leading to IκB phosphorylation. Phosphorylated IκB is rapidly degraded through ubiquitination, releasing the NF-κB dimers from their inhibitory proteins and activating NF-κB. The NF-κB complexes then enter into the nucleus to exert their biological effects. IκB is thus regarded as the key to activation of the NF-κB pathway\textsuperscript{28}. SAADIA BASHIR HASSAN\textsuperscript{16} found that alpha-terpinol could inhibit the proliferation of MM cell line RPMI-8226 by inhibiting NF-κB. In view of the important role NF-κB plays in myeloma, treatments targeting the NF-κB pathway have become a research focus for MM treatment in recent years. PDTC is an
inhibitor of the NF-κB pathway, which inhibits IκB phosphorylation, thus reducing its ubiquitination and degradation, and eventually inhibiting activation of the NF-κB pathway. In this study, the IκB level in RPMI-8226 cells was found to be significantly increased after the cells were treated with 25 µM PDTC for 24 h, indicating the NF-κB pathway was inhibited. Two groups, i.e. HK-2 alone culture group and HK-2 alone + PDTC culture group, were set up in this study to check interference of PDTC on HK-2 cells in the lower chamber. Flow cytometric and western blot analyses showed that addition of PDTC to the upper chamber in the HK-2 alone culture group did not cause any changes of apoptosis-associated proteins in the HK-2 cells. However, when PDTC was added to the upper chamber in the RPMI-8226/HK-2 co-culture group, the apoptotic rate of HK-2 cells was significantly reduced, and levels of the cell surface markers E-cadherin and α-SMA were reversed to some extent. In addition, secretion of κ light chain and λ light chain in RPMI-8226 cells was significantly reduced. These findings suggested that the NF-κB pathway in RPMI-8226 cells was inhibited by PDTC, leading to increased apoptosis, decreased secretion of light chain proteins, and less effect on HK-2 cell apoptosis and cell phenotype transformation. Interestingly, PDTC simultaneously reduced secretion of κ light chain and λ light chain without affecting their ratio. It was probably due to the fact that the RPMI-8226 cell line used in this study was a myeloma cell line that mainly secretes κ light chain. Some studies also found that when HK-2 cells were treated with RPMI-8226 cell culture medium, E-cadherin of HK-2 cells increased, while Vimentin decreased, which was not consistent with the results of this study. We speculate that it may be caused by some signal communication between the two kinds of cells in the co-culture system used in this study. In the culture medium of RPMI-8226 cells used in Aiqi Zhao's research, there was only one factor secreted by cells, lacking the signal molecule produced by the communication between the two cells.

Conclusion

In conclusion, PDTC inhibited activation of the NF-κB pathway in RPMI-8226 cells, leading to increased apoptosis, reduced secretion of light chain proteins, and decreased effect on apoptosis and cell phenotype transformation of HK-2 renal tubular epithelial cells.

Declarations

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Author contribution

XY designed the study and drafted the manuscript. JB and XC were responsible for the collection and analysis of the experimental data. FD and YW revised the manuscript critically for important intellectual
content. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval and consent to participate

The study was approved by the Ethics Committee of The Third Affiliated Hospital of Qiqihar Medical University, China. Signed written informed consents were obtained from the patients and/or guardians.

Consent for publication

Not applicable.

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Figures
Figure 1

Effect of PDTC on the cell activity of RPMI-8226 and HK-2 a. Effects of different concentrations of PDTC on RPMI-8226 cell activity at different time. b. Effects of PDTC on the cell activity of HK-2 in single culture and co-cultured systems. *p<0.05, compared with the HK-2 alone culture group; #p<0.05, compared with the RPMI-8226/HK-2 co-culture group.
Figure 2

Effect of PDTC on apoptosis of renal tubular epithelial cells HK-2 in each group. *p<0.05 compared with the single culture group; #p<0.05 compared with the co-culture group.
Figure 3

Western blot analyses of related proteins: a, b apoptosis-associated proteins; c, d cell surface marker proteins
Figure 4

Effect of PDTC on NF-κB pathway activation in RPMI-8226 cells in co-culture system. *p<0.05, compared with co-culture group