ENDOPLASMIC RETICULUM STIMULATION INDUCES APOPTOSIS OF ALVEOLAR EPITHELIAL CELLS IN COPD AND THE ANTI-APOPTOTIC EFFECT OF GRP78

Xiaodong Yang[1], Chenxu Zhao[1], Xuemei Ma[1], Shanzuan Wang[2,*]
[1]Department of Respiratory and Critical Care Medicine, the First Affiliated Hospital of Jiamusi University, Jiamusi 154002, PR China - [2]Department of Respiratory and Critical Care Medicine, Affiliated Hospital of Putian University, Putian 351100, PR China

ABSTRACT

Objective: *The purpose of this study is to reveal that endoplasmic reticulum stress is induced by alveolar epithelial cells of rats with chronic obstructive pulmonary disease (COPD), and that endoplasmic reticulum stress in COPD may be an important way to mediate the apoptosis of alveolar epithelial cells.*

Methods: *This study set up four experimental objects: COPD group, endoplasmic reticulum group, blank control group, and GRP78 group. Through the establishment of a COPD rat model, the endoplasmic reticulum stress markers GRP78, COPD, apoptosis morphology, and activated caspase-3 protein water were detected. Then, the lung function of rats was detected by H&E staining, and the levels of SOD and MDA in plasma were measured.*

Results: *The results indicate that the amount of SOD in endoplasmic reticulum stress-induced COPD was 15.30±0.62 u/ml, which was close to 10 u/ml. Compared with the blank control group, the content of the COPD group also decreased and the value range was 21.47±1.52 u/ml. Finally, there were 48 apoptotic cells in the endoplasmic reticulum group, followed by 38 in the COPD group and 33 in the blank control group.*

Conclusion: *Endoplasmic reticulum stimulation induces apoptosis of alveolar epithelial cells in rats with COPD and the anti-apoptotic effect of GRP78.*

Keywords: *Endoplasmic reticulum, alveolar epithelial cells, apoptosis rate, glucose regulatory proteins.*

DOI: 10.19193/0393-6384_2022_5_451

Received *March 15, 2021;* ***Accepted*** *April 20, 2022*

Introduction

Background and significance

Chronic obstructive pulmonary disorder (COPD) is a preventable respiratory disease that is usually progressive. Harmful gases and tobacco smoke can cause diseases in the trachea and alveolar tissues. According to the statistical data of the World Health Organization (WHO), COPD is expected to become the third leading cause of death in the world, and its financial burden will become the fifth highest among diseases. In China, it will likely become the most financially burdensome disease. Modern medicine has not yet discovered the pathogenesis of COPD, though it is generally related to lung inflammation and other factors.

The imbalances between oxidation and oxidation resistance and between protein and antiprotease loss are lost. Furthermore, the pathological manifestations are bronchitis and emphysema. Inflammation is characterised by excessive cell deposition in the lung structure, resulting in decreased lung function, increased residual lung volume, and increased resistance to ventilation tubes, which has become the main pathological manifestation.

Related work

Cell stress, especially the response to toxicity and metabolic damage that interferes with endoplasmic reticulum (ER) function, is associated with a powerful inducible factor chop. Wu studied the role of chop in endoplasmic reticulum stress injury in mice. Compared with the wild type, mouse embryonic fibroblasts (MEFs) from chop animals showed significantly less programmed cell death when stimulated by drugs that interfere with endoplasmic reticulum function.

Similar defects in programmed cell death induced by endoplasmic reticulum stress were observed in the major dimer C/EBPbeta lacking chop, suggesting that the chop-c/EBP pathway is involved in programmed cell death. Wu has established an animal model to study the effect of chop on endoplasmic reticulum stress response. This model requires a single subcutaneous injection of tunicamycin to mice with the identified chop genotype, causing a serious disease characterised by transient renal insufficiency[1]. Glucocorticoid is an effective anti-inflammatory drug that can affect the release of cytokines in a variety of cells. The purpose of this study was to investigate the effects of glucocorticoids commonly used in asthma treatment on cytokines secreted by pulmonary epithelial cells and alveolar macrophages.

Inhalation of cigarette smoke can cause airway inflammation, introduce a large number of inflammatory cells into the lung, and release pro-inflammatory cytokines. Therefore, the inhibitory effect of budesonide and fluticasone propionate on cytokine release was studied in a dose–response manner by stimulating human lung epithelial cells (A549) and alveolar macrophages with cigarettes or lipopolysaccharide (LPS), and the time course of hormone action was studied[2].

Innovation

The aim of this study was to investigate the effect of endoplasmic reticulum on apoptosis of alveolar epithelial cells in COPD and the anti-apoptotic effect of GRP78. Four groups of experimental objects were set up, and different experimental methods were used to detect the required data and compare the differences between the data to get the experimental results.

Endoplasmic reticulum stimulation mediates apoptosis of alveolar epithelial cells in COPD and grp78 inhibits apoptosis

Effect of endoplasmic reticulum on apoptosis

The endoplasmic reticulum is widely distributed in eukaryotic cells. This membrane organelle can provide sites for protein composition, lipid metabolism, and steroid hormone synthesis. It is also the main reservoir of $Ca2^{+}$ and helpful in maintaining the uniformity of the intracellular environment. The composition and secretion of proteins and the correct modification of the latest protein components, such as reproduction and oligomerisation, depend on the balance and stability of the endoplasmic environment[3]. Among them, chaperones – such as glucose regulatory protein 78 (GRP78), sulfhydryl oxidoreductase, and cadherin – are involved in the formation of protein conformation, depending on endoplasmic reticulum. When stimulated, the balance of the endoplasmic reticulum system is destroyed, which destroys the signal of protein synthesis and finally leads to endoplasmic reticulum stress (ERS) response[4]. Through the continuous expression of the degradation system associated with endoplasmic reticulum, a large number of duplicated proteins are degraded. When the system is degraded, the folding and degradation in cells are overloaded. A large number of proteins are widely folded and degraded, which has toxic effects on the body and causes cell decay, leading to cell apoptosis, which is conducive to maintaining the normal function of cells, protein composition, and the stable state of metabolism.

Some studies have confirmed that, during the onset of pulmonary fibrosis, the endoplasmic reticulum stress pathway is involved in epithelial cell degradation. However, in the process of endoplasmic reticulum stress, mitochondria are still the important organisms for cell apoptosis. Endoplasmic reticulum stress is a mechanism of cell self-protection. In the early stage, when cells are affected by harmful factors, abnormal protein accumulation may reduce cell damage by activating unfolded protein response (UPR). UPR is mainly carried out through protein signalling pathways, such as the perk-eif2a signalling pathway, ire1-xbp1 signalling pathway, and ATF6 signalling pathway. If the stress state of endoplasmic reticulum stress continues, these intracellular signals will change from promoting cell survival to promoting cell decay[5]. Continuous activation of chop can decrease the anti-rejection protein of Bcl-2 and activate the mitochondrial pathway regulated by Bax. Additionally, the calcium signalling pathway is involved in ers-chop-induced apoptosis. The realisation of the ultimate goal also depends on the morphological changes of the

mitochondrial permeability transition pore and the entry of the mitochondrial apoptosis pathway. UPR realises self-protection through ers. Due to the effects of hypoxia, oxidative stress, and other pathological factors, the aggregation of unfolded or misfolded proteins occurs in the endoplasmic reticulum.

When stress occurs, signals are transmitted to the nucleus through a specific signal pathway, affecting protein composition and degradation and triggering the series of self-protection chain reactions known as UPR[6]. The endoplasmic reticulum activates the UPR protection mechanism and plays an important role in repairing damaged cells, restoring normal cell function, purifying necrotic cells, and maintaining the stability of the bodily environment.

GRP78 structure

GRP78 was first detected in fibroblasts infected with avian meat virus and is a member of heat shock protein 70 (HSP70), also known as immunoglobulin and heavy chain binding protein. It is called GRP78 because its expression is opposite to glucose supply, and it is widely found in eukaryotic cells, including yeast and human somatic cells[7]. Human GRP78 gene, located on chromosome 9, consists of 7 introns and 8 exons, which encode 654 amino acids.

The structure of GRP78 can be divided into the following parts: Starting from the N-terminus, amino acids 1-18 are the signal peptides for endoplasmic reticulum positioning, which is the key to its important role in the endoplasmic reticulum. The 128-280 amino acids constitute the ATPase domain of GRP78. Every 4 helixes in this domain form a crack, and there is an ATP connection point at the bottom of each crack. When ATP is combined with it, GRP78 has ATPase activity[8]. The 100 amino acids at positions 400-500 are the substrate connection region, which mainly functions by binding to the polypeptide domain. When a protein enters the transport cell and escapes from the intracellular endoplasmic reticulum due to incorrect reproduction or packaging, the receptor connected to the membrane associated with the endoplasmic reticulum can accurately recognise the sequence and guide the protein to escape and remain in the endoplasm online.

Positioning and function of GRP78

GRP78 is an important molecular chaperone in the endoplasmic reticulum because it can be attached to the newly formed and folded peptides to help them refold and assemble. GRP78 is also a key regulator of the endoplasmic reticulum stress response, as it is connected with $Ca2^{+}$ and makes $Ca2^{+}$ in the endoplasmic reticulum to maintain stability[9]. In addition, GRP78 is widely expressed when cells are exposed to hypoxia and glucose deprivation, which is a sensor in the process of endoplasmic reticulum stress response.

In normal cells, GRP78 binds to some transmembrane receptors on the endoplasmic reticulum and blocks the activation of these signals. Due to the development of their respective vascular systems, abnormal cells like tumour tissues and cancer cells are usually in a hypoxic stress environment. In the absence of glucose and an acid stress environment, GRP78 will be disconnected from the related receptor, which will lead to the activation of ATF6, IRE1, and perk down signalling pathways, and will eventually lead to the abnormal expression and localisation of GRP78. The detection of GRP78 in cancer cells is not limited to the endoplasmic reticulum, as it has been expressed in the nucleus, mitochondria, cytoplasm, cell membrane, and extracellular regions. When cancer cells are under pressure, GRP78 may be required to enter the nucleus, which prevents cancer cells from undergoing apoptosis by binding with DNA and may promote GRP78 entering mitochondria[10]. While in the mitochondria, GRP78 helps maintain homogeneity and protects cancer cells from harsh environments. GRP78 with the wrong selective distribution is distributed in the cytoplasm, which could improve the survival rate of cancer cells under compression. On the surface of the cell membrane, it may be a receptor protein, which is related to the migration and reduction of tumour cells. In addition, when secreted by cancer cells, the protein may act as a communication molecule between cancer cells and stromal cell tumours.

Therefore, GRP78 may have different detection modes and functions in cancer cells. In the cells without endoplasmic reticulum stress, GRP78 could be connected to the three receptor proteins of the network membrane, Pei water, ATF6, and IRE1, with the last one being inactive. When endoplasmic reticulum stress occurs, GRP78 is separated from the receptor protein and connected to an unfolded protein in a muscle cavity to help protein reproduction. In the initial stage of endoplasmic reticulum stress, UPR can upregulate the expression of GRP78, GRP94, and other molecular chaperones, thus promoting protein reproduction. Apart from chaperone proteins, UPR can reduce protein folding

load and reduce unfolded or misfolded proteins through ER-related degradation. When the stress response of endoplasmic reticulum occurs, the protein or calcium ion unfolding in the endoplasmic reticulum is unbalanced, which leads to protein imbalance. Through unfolded protein reaction, the expression of serum molecular proteins, such as GRP78 and GRP94, can be upregulated to promote protein proliferation. Among them, the adaptability of GRP78 expression should stimulate the key protective effect on endoplasmic reticulum, contribute to the normal diffusion of unfolded and misfolded proteins, reduce the stress in the endoplasmic reticulum stress response, prevent the exposure of the endoplasmic reticulum membrane, and reduce the occurrence of apoptosis.

Apoptosis

COPD is a complex disease, and its pathological mechanism is still unclear. Moreover, COPD patients have different degrees of lung cell degradation and structural reconstruction, which indicates that the proliferation and death of lung cells in COPD patients are in an unbalanced state. Cell death usually takes two forms: necrosis and degeneration. Apoptosis refers to the planned natural death of cells by activating their internal mechanism under certain conditions. It is a method of regulating cell population balance by organisms, and its process is the opposite of mitosis. Under normal conditions, it can ensure the normal development of organisms and the smooth progress of the life process[11]. Under pathological conditions, apoptosis can lead to excessive or insufficient cell decay, thereby breaking the imbalance of cell population and leading to various diseases.

Apoptosis plays an important role in the pathogenesis of COPD, as it is closely related to the formation of the disease. The abnormal apoptosis of lung cells in COPD patients includes inflammatory cells with neutrophil apoptosis and lung parenchymal cells with airway epithelial cell apoptosis. The proliferation and decay of lung cells were carried out at the same time so as to maintain the stability of tissue quantity. The increase of neutrophils in the respiratory tract of COPD patients is not only related to inflammation and oxidative reaction, but also to neutropenia. Furthermore, tobacco contains nicotine, tar, aldehydes, and other harmful chemical substances, which may lead to cell epithelial cell decay. Type II alveolar epithelial cells are one of the main types of alveolar cells. They have important functions, such as ion transport, regulating surfactant metabolism, and repairing damaged lung tissue. AEC II continues to proliferate during lung development, which assigns it a defence function.

The excretion function can also be differentiated into AEC I, forming the Qi blood barrier and participating in gas exchange[12]. When the cell epithelium is damaged, AEC II differentiates the granules into AEC I to repair the damaged cell epithelium. When cells are destroyed, AEC II is the main decay cell.

The depletion of epithelial cells in COPD patients increases significantly, which further damages the structure of lung tissue and leads to the formation of COPD.

Endoplasmic reticulum stimulation mediated apoptosis of alveolar epithelial cells in COPD and GRP78 anti-apoptosis experiment

Research object

Eighty healthy male SD SPF rats (age: 5 weeks, weight: 190-210 g) were purchased from the animal experimental centre of a municipal medical college. After the rats were purchased, they were properly raised in the animal laboratory of our medical college and fed distilled water and a uniform diet. The experiment began two weeks later.

Experimental steps

Experimental animal grouping and model preparation

After proper feeding for two weeks, the eighty rats they were randomly divided into four groups of twenty: COPD group, endoplasmic reticulum intervention group, blank control group (control group), and GRP78 group.

COPD group: On days 1–10 and 11-20, the rats were put into a smoke box (30 cm × 50 cm × 20 cm) to smoke five cigarettes passively for half an hour (each cigarette contains 9 mg of tar and 2.0 mg of nicotine). Before smoking, rats were injected with normal saline, and 2% Pentobarbital Sodium (30 mg/kg) was injected intraperitoneally on days 1 and 7. After loosening their limbs, the rats were fixed on the rat plate and the hair in the front area of the neck was cut off with scissors so that the neck could be fully exposed. The neck skin of rats was disinfected with iodine and carefully cut along the trachea.

The rats' muscles were evenly separated with a haemostatic clip and the trachea was fully exposed.

Then, the trachea was punctured with a 2 ml empty needle and 100 ml of lipopolysaccharide solution prepared beforehand was injected. After injection, in order to evenly distribute the lipopolysaccharide solution in both lungs, the rat plate was raised and moved left and right several times. Additionally, the wound was sutured and disinfected in a timely manner.

• Endoplasmic reticulum group: the experimental method was the same as that of the COPD group, but the difference was that endoplasmic reticulum stress was mediated for 15 minutes a half hour before passive smoking on days 1–10 and 11–20.

• Control group: on the first and tenth day, the same dose of saline was injected into the trachea (the procedure was the same as the cap group), and the rats were placed in the smoke box to breathe air on days 1–10 and 11–20.

Specimen collection and treatment

The anaesthetic dose of 1% Pentobarbital Sodium was calculated with 15 mg/kg and intraperitoneal injection was performed. When their limbs were relaxed, the rats were fixed on the rat plate, usually disinfected with iodine, and cut along the midline of the abdomen.

Then, blood was taken from the abdominal aorta, put into the LEP tube, and centrifugated in a low-temperature centrifuge for 10 minutes. The supernatant was stored in a refrigerator at -15°C to detect the contents of SOD and MDA. Additionally, the thoracic cavity was opened with gentleness to avoid lung injury. The lung lobes were separated with a cotton swab, the trachea was exposed, the right main bronchus was tightened, the left lung was flushed with 1 ml of normal serum, bronchoalveolar lavage fluid (BALF) was recovered 5 consecutive times, and mucus was filtered with a sterile gauze. All recovered BALF was transferred to a sterile cup and stored in a refrigerator at -15°C for testing. After cleaning the bronchial cells, both lungs were removed, washed with normal saline, and then dried with gauze.

The right upper lobe was isolated and placed in newly prepared 1% neutral formaldehyde for 12 hours. Then, the tissue was dehydrated with ethanol and embedded in paraffin. After preparation, H&E staining, immunohistochemistry, and detection of alveolar epithelial cells were performed. All experiments involving the rats were in line with animal ethics.

Index detection

Cell count and classification of alveolar lavage fluid

The BALF was measured and classified with a blood cell counting board. The recovered BALF was then centrifuged at 0°C for 5 minutes at 1000 rpm. The supernatant was discarded and precipitated with 1 ml PBS (1% BSA); 10 ul of the supernatant was drawn and counted on the cell counting plate. The remaining liquid was centrifuged again, and most of the supernatant was discarded. After air drying, the supernatant was fixed with 50% ethanol. Wright's staining was used to count and classify the cells, and the ratio of 300 neutrophils to macrophages was determined.

Analysis of immunohistochemical results

The yellow-brown expression of the related proteins was detected by immunohistochemistry. Four visual fields were selected from each slide, and the visual field was 500 times of the microscope light. The integrated optical density value positive cells were measured by a computer image analysis system to express the intensity of protein positive expression. Then, the chop and caspase-3 of rats in each group were compared.

Cell transfection

The well-growing alveolar epithelial cells and GRP78 cells were washed twice with PBS solution and then evenly spread on the five-well plate after digestion. When the cells adhered to the wall and increased to 50–70% of the lower surface, transfection was performed.

Before transfection, 1200 m of fresh culture medium without antibiotics was switched. Then, an aseptic EP tube was added with 400 μl of serum-free medium, 4 μg of plasmid DNA, and 6 μg of transfection reagent. After being placed at room temperature for 15 minutes, it was evenly added into 5 well plates, slightly shaken, and cultured in a constant temperature incubator for 3 hours.

Statistical treatment

Statistical software was used to test the relevant data, analysis of variance was used to compare multiple sample groups, and an LSD test was used to compare pairing within groups. The experimental results were expressed as mean ± standard deviation ($x \pm s$); $P<0.05$ means the difference is statistically significant.

$$A_n = \frac{a_1 + a_2 + ... + a_n}{n} \quad (1)$$

In the above formula, a represents each number, n represents the number of numbers, and A represents the average value.

The formula of standard deviation is as follows:

$$s = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n-1}} \quad (2)$$

where X is each number, $\bar{X}$ is the average value, n is the number of numbers, and s is the standard deviation.

Endoplasmic reticulum mediated apoptosis of alveolar epithelial cells in COPD and the anti-apoptotic effect of GRP78

Determination results of SOD and MDA in rat plasma

In the early stages of modelling, the rats with COPD preferred to congregate in smoking crates. In the middle and late stages of modelling, they began to suffer from anorexia, weight loss, reduced activity, light fur, shortness of breath, shortness of breath accompanied by obvious wheezing, and slow action response. Some rats exhibited symptoms of drowsiness, irritability, and asthma. During the second airway drip of lipopolysaccharide, one rat in the COPD group died, likely due to pulmonary infection or anaesthesia overdose. The rats in the control group experienced healthy appetites, weight gain, rapid reaction, bright fur, and pink lips and limbs. Throughout the experiment, the rats in the endoplasmic reticulum group generally performed better than those in the COPD group, as shown in Table 1.

Group	MDA (nmol/L)	SOD (U/ml)
Control group	4.26 ± 0.8	25.13 ± 0.89
Endoplasmic reticulum	7.26 ± 0.61	15.30 ± 0.62
COPD group	5.42 ± 1.02	21.47 ± 1.52

Table 1: Contents of SOD and MDA in the plasma of rats in each group.

As shown in Table 1, the contents of SOD and MDA in the three experimental groups were different. Among them, the content of MDA in COPD mediated by endoplasmic reticulum stress was the highest with the range of 7.26±0.61 nmol/l, while that of the COPD group was 5.42±1.02 nmol/l, which itself was higher than that of the blank control group. Moreover, the content of endoplasmic reticulum in the experimental group was significantly higher than in the control group (P<0.05). Compared with the blank control group, the content of endoplasmic reticulum in the COPD group also decreased, with a range of 21.47±1.52 u/ml. In order to clearly distinguish the difference in plasma between the different groups, a histogram was made. The data in the graph were taken as the average value, as shown in Figure 1.

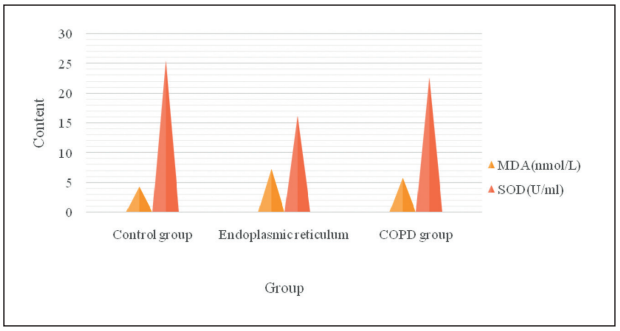

Figure 1: Changes of SOD and MDA in the plasma of rats in each group.

The activity and level of SOD was significantly lower in the COPD group than in the control group (P<0.05). Additionally, the endoplasmic reticulum group had higher MDA activity and lower MDA levels than the COPD group (P<0.05).

Comparison of lung function test results of rats

The rats in the blank control group had clean and bright hair, no restlessness, stable breathing, wet noses, demonstrated stable eating and drinking, normal activities, and weight gain. In the COPD group, the wound healed after 7 days. After each smoking session, the rats exhibited a shortness of breath, increased body temperature, congested eyes, red noses, and increased secretion. Furthermore, throughout the whole modelling process, the rats in the COPD group demonstrated initial irritability, mental depression, withered and dull hair, yellow, increased water intake, reduced activity, dry stool, and a tendency to either curl up or gather with others. The general situation of the endoplasmic reticulum group was between the blank control group and COPD model group in terms of severity.

As shown in Figure 2, at the end of two weeks, the pulmonary function of rats in the COPD group was detected. Compared with the blank control group, fev0.3/fvc (%) of rats in the COPD group increased and airway resistance (RI) showed a downward trend, which proved that the model group

was consistent with the changes of COPD pulmonary function. The multiple comparison differences in fev0.3/fvc and RI of the 20 groups were statistically significant ($P<0.05$). Finally, the situation of the endoplasmic reticulum group was between the blank control group and COPD model group in terms of severity.

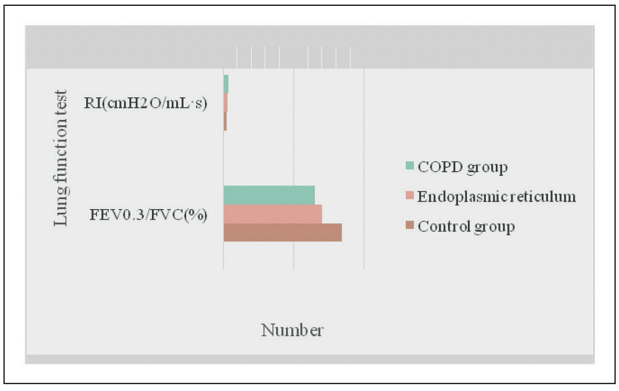

Figure 2: Comparison of lung function in rats.

Detection after dyeing

Apoptosis is a natural death process that develops in a stable and controllable manner according to a specific, predetermined procedure. It is based on a series of mechanisms to activate, express, and regulate genes. This procedure is also closely related to the normal development of the body, the occurrence of some diseases, and the malignant transformation of cells. Due to the fact that past studies have shown that the imbalance of apoptosis will cause direct damage to lung tissue, the role of apoptosis in the pathogenesis of COPD has been given more attention recently.

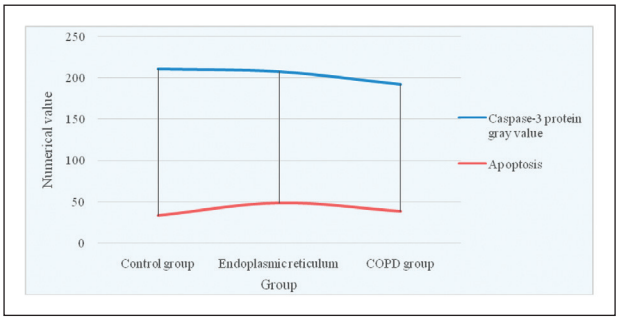

Figure 3: Comparison of apoptosis detection results.

As shown in Figure 3, TUNEL staining demonstrated that the brown granular cells in the nucleus were positive for TUNEL cells. There was also a significant difference in apoptosis rates between the COPD group and control group; the number of apoptotic cells in endoplasmic reticulum group was the highest (48), followed by the COPD group (38) and blank control group (33 cells). The positive protein of Caspase-3 was brown or yellowish-brown when observed by ordinary microscope. Compared with the blank control group, the grey value of lung tissue in the COPD model group decreased significantly, and there was no significant difference between the endoplasmic reticulum group and blank control group.

Endoplasmic reticulum stress mediates GRP78 anti-apoptosis

The endoplasmic reticulum is the main site for protein processing, Ca2 + storage, and lipid metabolism. Its main function is to fold and transport new proteins. When the function of the endoplasmic reticulum is disordered, proteins cannot be folded and processed normally and a large number of misfolded or unfolded proteins accumulate in the endoplasmic reticulum cavity, which leads to the endoplasmic reticulum stress response. When cells are injured by acute or chronic low-level injury, endoplasmic reticulum stress reaction occurs in cells and various signal pathways are activated to make the unfolded or misfolded protein correctly fold or no longer increase to restore the normal state of cells.

This phenomenon, known as UPR, is an adaptive response in vivo that promotes the normal function of cells. UPR signalling pathways include RNA-dependent protein kinase like the endoplasmic reticulum kinase signalling pathway, the activator of transcription factor signalling pathway, and the inositolase requirement pathway. Under normal circumstances, the three proteins are not activated by binding with the chaperone glucose regulatory protein. When endoplasmic reticulum stress occurs, however, a large number of unfolded proteins are produced and bound to GRP78. Three transmembrane proteins dissociate and activate GRP78, thereby activating downstream signalling pathways and mediating cell survival and apoptosis. The activation of the UPR signalling system plays an important role in the recovery of the normal function of stress cells. In order to analyse whether endoplasmic reticulum stress inhibited GRP78 cell apoptosis, the effect of the endoplasmic reticulum stress inhibitor on GRP78 cell apoptosis was detected, as shown in Figure 4.

GRP78, which is a molecular chaperone mainly located in the endoplasmic reticulum, is an important factor in cell stress. In cancer cells, its different distribution plays an important role in the survival, proliferation, malignant transformation, and angiogenesis of cancer cells. At the same time, GRP78 is an effective therapeutic target in cancer cells.

According to the data in the figure, there were 192.21 apoptotic cells in the GRP78, 210.41 in the control group, and 163.32 in the endoplasmic reticulum group. Thus, it can be seen that endoplasmic reticulum stress can inhibit the apoptosis of GRP78 cells.

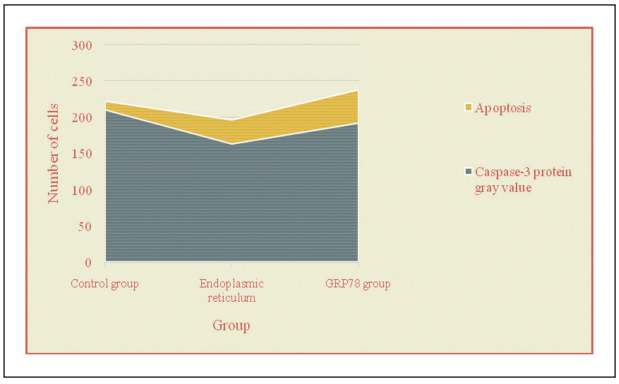

Figure 4: Comparison of anti-apoptosis of GRP78 cells.

Conclusions

Recent evidence has shown that the occurrence and development of COPD are closely related to cytopenia, especially regarding the condition and role of alveolar epithelial cells in cell decay during the formation of emphysema. Studies have shown that apoptosis is regulated by complex signalling pathways. At present, there are three main signalling pathways: the mitochondrial pathway, the death receptor pathway, and the endoplasmic reticulum stress-induced cell death pathway. For a long time in the model of cigarette-induced lung disease, epithelial cell abscission was mainly considered to be caused by the death receptor pathway of the mitochondrial pathway. Since cigarette smoke is rich in oxides and toxic components, oxidative stress and toxic substances can be used as endoplasmic reticulum inducers.

In this study, changes in GRP78 and COPD markers and proteins in rats' lung tissue were detected. The expression level of GRP78 in the lung tissue of normal rats was very low, while that of the rats with COPD was significantly increased. The results of immunohistochemistry indicate that GRP78 expression was the most obvious in the epithelial cells, suggesting that endoplasmic reticulum stress exists in lung tissue, especially in epithelial cells of COPD. The TUNEL method was used to detect the decrease of epithelial cells in rats with COPD. At the same time, changes in caspase-3 protein levels mediated by endoplasmic reticulum stress were detected. Thus, endoplasmic reticulum stress may be a mediating factor that can induce apoptosis of alveolar epithelial cells. When the expression of GRP78, the main molecule of endoplasmic reticulum stress, did not change, it could enter the cell membrane. It is an important research achievement that GRP78 can promote the transformation of cancer cells. In addition, the increased expression of GRP78 on the surface of cancer cells may lead to cancer cell death. Endoplasmic reticulum stress can promote the metastasis of GRP78 membrane to cancer cells and inhibit its degradation.

References

1) Chang JH, Lee YL, Laiman V, Han CL, Jheng YT, et al. Air Pollution-Regulated E-Cadherin Mediates Contact Inhibition of Proliferation via the Hippo Signaling Pathways in Emphysema. Chem Biol Interact 2022; 351.
2) Guan R, Yao H, Li Z, Qian J, Yuan L, et al. Sodium Tanshinone IIA Sulfonate Attenuates Cigarette Smoke Extract-Induced Mitochondrial Dysfunction, Oxidative Stress, and Apoptosis in Alveolar Epithelial Cells by Enhancing SIRT1 Pathway. Toxicol Sci 2021; 183(2): 352-62.
3) Retraction: Protective Effect of Autophagy on Endoplasmic Reticulum Stress Induced Apoptosis of Alveolar Epithelial Cells in Rat Models of COPD. Biosci Rep 2021; 41(6).
4) Retraction: Protective Effect of Autophagy on Endoplasmic Reticulum Stress Induced Apoptosis of Alveolar Epithelial Cells in Rat Models of COPD. Biosci Rep 2021; 41(6).
5) Li N, Li S, Wu Y, Xiong L, Li T, et al. Dexmedetomidine Targets miR-146a and Participates in the Progress of Chronic Obstructive Pulmonary Disease In Vivo and In Vitro. Genes Genomics 2021; 43(12): 1371-9.
6) Michaeloudes C, Li X, Mak JCW, Bhavsar PK. Study of Mesenchymal Stem Cell-Mediated Mitochondrial Transfer in In Vitro Models of Oxidant-Mediated Airway Epithelial and Smooth Muscle Cell Injury. Methods Mol Biol 2021; 2269: 93-105.
7) Yang YK, Li Y, Wang YY, Ruan GP, Tian C, et al. The Effects of BMMSC Treatment on Lung Tissue Degeneration in Elderly Macaques. Stem Cell Res Ther 2021; 12(1): 156.
8) Yuan D, Liu Y, Li M, Zhou H, Cao L, et al. Senescence Associated Long Non-Coding RNA 1 Regulates Cigarette Smoke-Induced Senescence of Type II Alveolar Epithelial Cells Through Sirtuin-1 Signaling. J Int Med Res 2021; 49(2).

9) Yan M, Wu Y, Peng W, Fu C, Giunta S, et al. Exposure to Particulate Matter 2.5 and Cigarette Smoke Induces the Synthesis of Lipid Droplets by Glycerol Kinase 5. Clin Exp Pharmacol Physiol 2021; 48(4): 498-507.
10) Wen X, Zhang H, Xiang B, Zhang W, Gong F, et al. Hyperoxia-Induced miR-342-5p Down-Regulation Exacerbates Neonatal Bronchopulmonary Dysplasia via the Raf1 Regulator Spred3. Br J Pharmacol 2021; 178(11): 2266-83.
11) Nouws J, Wan F, Finnemore E, Roque W, Kim SJ, et al. MicroRNA miR-24-3p Reduces DNA Damage Responses, Apoptosis, and Susceptibility to Chronic Obstructive Pulmonary Disease. JCI Insight 2021; 6(2).
12) Conlon TM, John-Schuster G, Heide D, Pfister D, Lehmann M, et al. Inhibition of LTβR Signalling Activates WNT-Induced Regeneration in Lung. Nature 2020; 588(7836): 151-6.

Acknowledgement:
Scientific Research Project of Heilongjiang Health Commission, Expression of UCP2 in COPD patients and its correlation with T lymphocyte subsets, (No. 2019-298).

Corresponding Author:
Shanzuan Wang
No.999 Dongzhen East Road, Licheng District, Putian City, Fujian Province, China
Email: pki6yu@163.com
(China)