Programmed cell death in the epithelial cells of the nasal mucosa in allergic rhinitis

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April 1, 2022

Abstract

Abstract In allergic rhinitis (AR), the epithelial barrier composed of nasal mucosal epithelial cells is the first line of defense, which is crucial to protect the host immune system from harmful stimuli. Moreover, irreversible structural changes in nasal mucosal epithelial cells can occur in response to different allergens, but the mechanism leading to such abnormal changes has not been determined. Programmed cell death is regulated by genes and interacts with multiple cell signaling pathways. To explore the regulatory mechanism and signal pathway of programmed cell death in epithelial cells of allergic rhinitis, is helpful to clarify the pathogenesis of AR and put forward treatment strategies. In this paper, the regulation mechanisms of programmed cell death such as apoptosis, pyroptosis, and autophagy occurring in epithelial cells in AR, are retrospectively summarized to better understand the pathogenesis of AR. FIG. 1 Programmed cell death in AR epithelial cells

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Running title

PCD in epithelial cells in AR

Acknowledgement

This work was supported by grants from Health Department of Jilin Province Project (JLSWSRCZX2021-092), Science and Technology Department of Jilin Province Project (20210402012GH)

Abstract

In allergic rhinitis (AR), the epithelial barrier composed of nasal mucosal epithelial cells is the first line of defense, which is crucial to protect the host immune system from harmful stimuli. Moreover, irreversible structural changes in nasal mucosal epithelial cells can occur in response to different allergens, but the mechanism leading to such abnormal changes has not been determined. Programmed cell death is regulated by genes and interacts with multiple cell signaling pathways. To explore the regulatory mechanism and signal

pathway of programmed cell death in epithelial cells of allergic rhinitis, is helpful to clarify the pathogenesis of AR and put forward treatment strategies. In this paper, the regulation mechanisms of programmed cell death such as apoptosis, pyroptosis, and autophagy occurring in epithelial cells in AR, are retrospectively summarized to better understand the pathogenesis of AR.

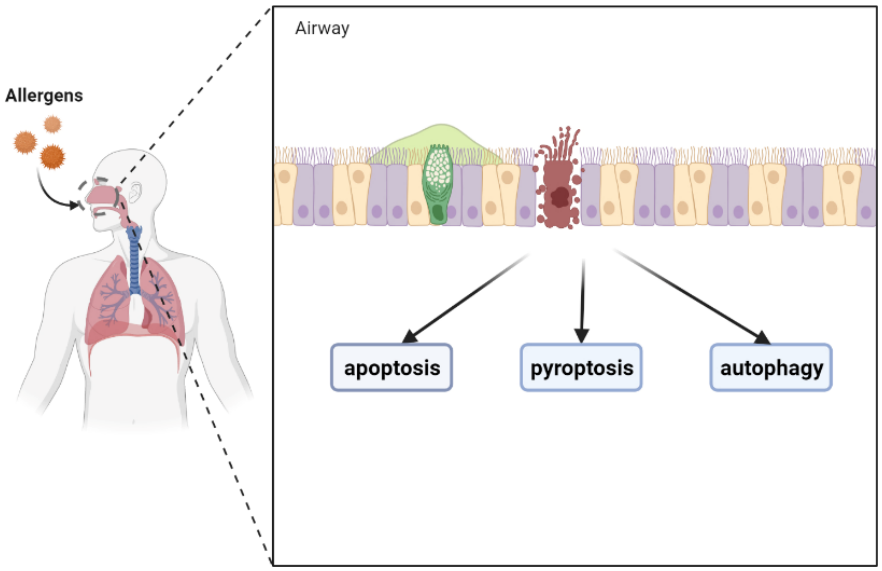

FIG. 1 Programmed cell death in AR epithelial cells

Introduction

Among allergic diseases, allergic rhinitis (AR) has a high incidence, and its main symptoms include nasal congestion, runny nose, sneezing, and itchy nose [1]. Chronic inflammatory infiltration leads to nasal mucosa swelling in AR patients, impeding ventilation and seriously affecting activities, sleep, and quality of life. Factors that induce AR mainly include airborne pollen, mold, dust mites, etc.[2]. Previous studies on the pathogenesis of AR have mainly focused on inflammatory cells and reactions in nasal mucosal tissues, while less attention has been paid to studies on nasal epithelial cells, which are the first line of defense against allergen infiltration. The natural barrier of the nasal mucosa is the first line of defense in the respiratory tract against foreign microorganisms, allergens, and various pollutants [3]. As a physical, chemical, and immune barrier in the nasal cavity, the epithelial cells of the nasal mucosa play an important role in resisting allergens, pathogens, and other foreign particles. Abnormal changes in nasal mucosal epithelial cells have been found in studies of different allergens. Cell death is an essential process for maintaining normal tissue and body function. When a cell is severely damaged, its function and structure will undergo irreversible changes, namely cell death. Accidental cell death (ACD) and regulated cell death (RCD) can be classified according to whether the process is controllable or not. In physiological conditions, RCD is also called programmed cell death. There are many modes of programmed cell death. Currently identified are apoptosis, ferroptosis, necroptosis, pyroptosis, parthanatos, autophagy, entosis, lysosome-dependent cell death, NETosis, oxeiptosis, and alkaliptosis [4].

Apoptosis in AR epithelial cells

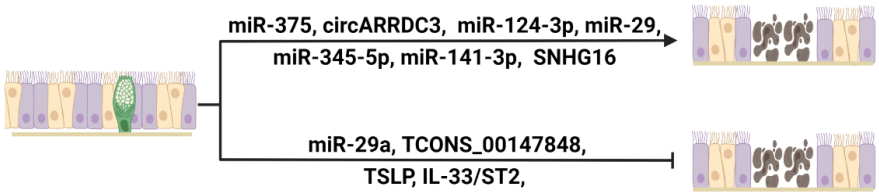

Figure 2. Promoting/inhibiting effects of different molecules on apoptosis of AR epithelial cells

The entry of healthy cells into apoptosis is determined by signaling. First, cells receive and respond to death signals and then undergo a death process such as nuclear pyknosis, cell shrinkage, cell membrane blistering, or DNA fragmentation. All cells are self-cleaved and activated by programmed death signals mediated by the suicide protease family (caspase), which in turn activates other members of the family and causes a cascade of protein enzymolysis. In addition, different caspases correspond to different activation signals; for example, caspase-3, 6, 7, and 8 play a role in Fas/TNF-mediated apoptosis. Caspase-9 and 3 participate in apoptosis mediated by APAF-I and cytochrome C in mitochondria.

At present, there are two views on the role of apoptosis of epithelial cells in AR. Some researchers believe that apoptosis of nasal mucosal epithelial cells in AR leads to destruction of epithelial barrier function and exacerbates the development of AR. This part of the study mainly focuses on the role of miRNA in apoptosis of AR epithelial cells. The expression of miR-375 was decreased in nasal epithelial cells and TNF-α-stimulated nasal epithelial cells of AR in mice, while its target gene JAK2 expression was increased. Thus, miR-375 prevents apoptosis of nasal mucosal cells and alleviates AR by inhibiting the JAK2/STAT3 pathway [5]. CircARRDC3 negatively regulates its expression by targeting the miR-375/KLF4 axis and then upregulates the expressions of GM-CSF, eotaxin, and MUC5AC, promoting the development of AR and epithelial cell apoptosis [6]. It has been reported that miR-124-3p was downregulated in AR nasal mucosal tissues, and overexpression of miR-124-3p reduced the frequency of nasal rubbing and sneezing, the number of eosinophils, and the apoptosis of nasal mucosal epithelial cells in mice with AR [7]. Decreased expression of miR-29 was observed in AR, and overexpression of miR-29 relieved allergic symptoms in mice with AR and significantly reduced the concentration of ovalbumin (OVA)-specific IgE, IL-4, IL-6, IL-10, and IFN-γ levels. Alleviating pathological changes in nasal mucosa reduces eosinophil infiltration and reduces apoptosis of nasal epithelial cells[8]. In addition, miR-345-5p plays an important role in alleviating inflammation and inhibiting apoptosis and fibrosis of nasal epithelial cells by inhibiting TLR4/NF-ϰB pathway in mice with AR[9]. It was found that miR-141-3p was significantly downregulated in nasal tissues of patients with AR. Overexpression of miR-141-3p can target HMGB1 to inhibit lPS-induced mucin MUAC5AC production and apoptosis of nasal mucosal epithelial cells[10]. The expression of long non-coding RNA (lncRNA) SNHG16 in AR is upregulated and binds to miR-106b-5p. Activation of the JAK1/STAT3 signaling pathway by upregulation of leukemia suppressor factor (LIF) promotes epithelial apoptosis and inflammation in AR cells[11].

Other researchers reported that the apoptosis of epithelial cells decreased and abnormal hyperplasia occurred in AR. Some researchers found that perennial AR may be related to the decreased apoptosis of nasal mucosal cells [12]. Allergic epithelial cell apoptosis was significantly reduced, which may explain the increased epithelial thickening observed in some study participants[13]. The occurrence of AR is related to abnormal apoptosis of nasal epithelial cells. Reduced apoptosis of epithelial cells leads to hypertrophy of nasal mucosa, which induces nasal congestion, glandular hypersecretion, and other typical clinical symptoms [14]. Compared with healthy control group, the expression of Mir-29a was upregulated in nasal tissues of AR patients while the expression of its target gene FOS mRNA was downregulated. Overexpression of Mir-29a or FOS silencing in nasal mucosal epithelial cell lines RPMI2650 and HNEpC could promote cell proliferation and inhibit cell apoptosis [15]. TCONS_00147848 is highly expressed in nasal mucosa of AR patients. The expression of IgE, IL-4, IL-5, IL-9, and IL-10 in serum of mice was significantly higher than that of the control group, and TCONS_00147848 depression can reduce their expression. TCONS_00147848 promotes the proliferation of

nasal mucosal epithelial cells and inhibits their apoptosis by targeting FOSL2 and activating the JAK/STAT3 signaling pathway [16] The expressions of TSLP, IL-33 and ST2 in nasal mucosa epithelial cells of AR rats were significantly higher than those of control rats, and hypoxia further promoted their expression. Overexpressed TSLP and IL-33/ST2 promote cell proliferation, inhibit apoptosis, and enhance cell migration [17].

Studies are focused on the role of non-coding RNA in AR in epithelial apoptosis, but the caspase causing abnormal epithelial cell apoptosis needs to be clarified to determine the specific role of apoptosis in the AR epithelium.

Pyroptosis in AR epithelial cells

Pyroptosis is a type of programmed cell death that has been studied more recently. It is characterized by continuous swelling of cells until the rupture of the cell membrane, resulting in the release of cell contents and then activating a strong inflammatory response. In 2015, Shao Feng et al. [18] found that caspase-1 and caspase-11/4/5 are induced by the cleavage of a protein called gasdermin-D (GSDMD). GSDMD releases its N-terminal structure after being cleaved by caspase-1 or caspase-11/4/5. This domain has the activity to bind membrane lipids and punch holes in the cell membrane to release inflammatory mediators IL-1β, IL-18, and LDH. Changes in cell osmotic pressure lead to cell swelling and the eventual rupture of the cell membrane[19]. Pyroptosis is an important natural immune response, which plays an important role in antagonizing infection and receiving endogenous danger signals. Pyroptosis is widely involved in the occurrence and development of infectious diseases, nervous system-related diseases and atherosclerotic diseases, etc. In-depth study of pyroptosis is helpful to understand its role in the occurrence, development, and outcome of related diseases.

A small number of investigators have focused on the role of pyroptosis in AR epithelial cells, with increased IL-1β production and inflammasome activation found in both AR patients and mice. The results of animal experiments showed that NLRP3 knockout significantly inhibited the progression of AR, the inflammatory response of AR mice was reduced, and the scoria of epithelial cells was weakened. In addition, inhibitors of caspase-1 improved AR development in vivo. Mechanistically, NLRP3 inflammasomes promote the development of AR by enhancing the inflammatory response and pyroptosis of epithelial cells. Increased inflammation and nasal mucosal damage during AR are partly due to the accumulation of Apoptosis-associated speck-like protein containing a CARD (ASC) spots and scoria of epithelial cells [20]. In nasal and OVA-induced AR mouse models of AR patients, cell pyroptosis -related biomarkers (NLRP3, ASC, IL-1β, and IL-18) and pro-inflammatory cytokines (OVA-specific IgE, TNF-α, IL-4, and IL-5) were upregulated. Using ceRNA network analysis and cell assay, a hsa_circ_0000520 / miR-556-5p axis regulating NLRP3 in nasal epithelial cell lines was screened and verified. The results showed that both hsa_circ_0000520 knockout and miR-556-5p overexpression inhibited NLRP3-mediated pyroptosis and attenuated inflammatory response in mouse AR models[21]. PM 2.5 aggravates rhinitis symptoms, promotes serum IgE secretion, and damages nasal mucosal ultrastructure. In this process, NLRP3, caspase-1, GSDMD and IL-1β protein expression are significantly increased. NLRP3 /caspase-1/GSDMD-mediated pyroptosis is involved in the intensification of AR [22].

There has been few research focused on the epithelial cells of AR contrasting to asthma and other allergic diseases. During the process of pyroptosis, epithelial barrier function is damaged while inflammatory factor are released at the same time. However, the role of pyroptosis in AR epithelial cells should still be continue be researched.

Autophagy in AR epithelial cells

Autophagy refers to the process in which cells wrap misfolded proteins and damaged components with autophagic vesicles and transfer them to lysosomes for degradation. Autophagy pathways play important roles in cell, tissue, and organism homeostasis and are mediated by evolutionarily conserved autophagy-related genes (ATG). There is a clear etiological link between mutations in genes that control autophagy and human diseases, particularly neurodegenerative diseases, inflammatory diseases, and cancer. Autophagy selectively targets dysfunctional organelles, intracellular microorganisms, and disease-causing proteins, and defects in this process may lead to disease [23]. Autophagy, as an earlier identified mode of programmed

cell death, has been found to be associated with the occurrence and development of a variety of diseases, including infection, cancer, neurodegeneration, cardiovascular disease, and aging [24].

There is a correlation between autophagy and airway remodeling in AR. Autophagosome and autophagy markers are highly expressed in the upper airway of AR patients, beclin-1 mRNA level is increased, and beclin-1, LC3-II, and collagen III protein expressions are higher. Autophagy is also associated with corresponding changes in airway remodeling markers[25]. After agomir-338-3p was administered in an AR rat model, the expression of autophagy-related proteins was decreased and nasal symptoms were relieved. Overexpression of UBE2Q1 in RPMI-2650 attenuated the inhibitory effect of miR-338-3p on PM2.5 and induced autophagy through the AKT/mTOR pathway. miR-338-3p acts as an inhibitory factor in AR, while PM2.5 could exacerbat the process of autophagy[26]. Derp1 exposure enhances epithelial autophagy and impairs barrier function in epithelial cells. The reason is that Derp1 exposure increases the expression of miR-125b by increasing CXCR4 expression and activation and downregulates FoxP3 expression, leading to enhanced autophagy and an impaired epithelial barrier. In vivo analysis confirmed the role of CXCR4/ miR-125b /FoxP3 axis in the impaired epithelial barrier in AR [27].

Some studies have also reported the role of immune imbalance and autophagy in AR [28, 29], and analyzed the relationship between AR and autophagy from various perspectives. Although autophagy was discovered earlier, knowledge of the specific mechanism of autophagy and AR development in epithelial cells is still incomplete.

Other roles of programmed cell death in epithelial cells

Other programmed cell death includes necroptosis, ferroptosis, dependent cell death, alkaliptosis, oxeiptosis, NETosis, entosis, etc., but there are few reports related to AR. Among them, necrotic apoptosis and ferroptosis have been found in the epithelial cells in asthma, and dependent cell death has also been found to be related to the onset of lung diseases. These three programmed cell death modes are likely to play a role in the occurrence and development of AR.

Necroptosis is a type of PCD with necrotic characteristics, including cell membrane rupture, swelling of organelles, and an inflammatory response. Necroptosis of airway epithelial cells plays an important role in exacerbating airway allergic inflammation and may lead to exacerbation of asthma caused by respiratory virus infection. Necroptosis of airway epithelial cells led to exacerbation of asthma in a mouse model of allergic inflammation induced by house dust mites[30].

In the process of ferroptosis, there are no signs of cell apoptosis such as chromatin condensation, but it is accompanied by mitochondrial shrinkage and accumulation of lipid oxides. A large amount of lipid is produced in the catalysis of ferroptosis, which destroys the REDOX metabolic reaction in cells and eventually leads to cell death. Also, in the relevant studies of asthma, the co-localization levels of PEBP1 and 15-LO in human asthmatic airway epithelial cells (HAECs) were higher than those in normal patients, suggesting that ferroptosis may occur in asthmatic airway epithelial cells, and inhibition of ferroptosis in HAEC is an effective treatment for asthma [31].

Parthanatos is a novel PCD activated by PARP-1. Activation of PARP-1 promotes the release of apoptosis-inducing factor (AIF) from mitochondria and the transfer of AIF into the nucleus, resulting in DNA breakage and cell death. It does not depend on the caspase family proteins, and caspase inhibitors cannot prevent its occurrence. Currently, although no systematic studies have been reported in allergic diseases, the destruction of the epithelial barrier mediated by caspase may also be involved in the occurrence and development of related diseases. Smoke-mediated parthanatos pathway activation was increased in human bronchial epithelial (HBE) cells from habitual smokers compared to nonsmokers. This suggests that chronic smoke exposure leads to increased activation of the smog-mediated parthanatos pathway and hints at its role in the pathogenesis of smog-related lung disease[32].

Studies of other programmed cell deaths, such as alkaliptosis, oxeiptosis, NETosis, and entosis, are rarely reported in allergic diseases and may provide a new direction for future AR research.

Conclusion

In the study of AR, many researchers focus on mast cells, dendritic cells, T cells, innate lymphocytes, eosinophils, basophils and other immune cells and their complex interactions while ignoring the important role and function of epithelial cells in this process. The above conclusions indicate that apoptosis, pyrotopia, and autophagy in AR epithelial cells also play an important role in the development of AR, but the specific mechanism is still not fully understood. Investigation of these types of programmed cell death may be a new direction for future AR research.

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