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The role of PTEN in allergic inflammation

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Summary

Bronchial asthma is a chronic inflammatory disease of the airways, characterized by airway eosinophilia, goblet cell hyperplasia with mucus hyper-secretion, and hyper-responsiveness to inhaled allergens and to non-specific stimuli. Eosinophil accumulation and subsequent activation in bronchial tissues play critical roles in the pathophysiology of bronchial asthma. Many inflammatory mediators attract and activate eosinophils via signal transduction pathways involving an enzyme phosphatidylinositol 3-kinase (PI3-kinase). Studies using wortmannin, a specific inhibitor of PI3-kinase, have revealed the involvement of PI3-kinase in the biochemical transduction of activation signals generated by many inflammatory mediators in eosinophils. Wortmannin prevents the development of airway inflammation, either by inhibiting the eosinophil infiltration of bronchial tissues or their activation on arrival. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is part of a complex signaling system that affects a variety of important cell functions. PTEN opposes the action of PI3-kinase by dephosphorylating the signaling lipid phosphatidylinositol 3,4,5-triphosphate. Recently we have demonstrated that PTEN expression is diminished in airway epithelial cells of antigen-sensitized and -challenged mice. Administration of PI3-kinase inhibitors or adenoviruses carrying PTEN complementary DNA remarkably reduces eosinophil levels and inflammation. One likely mechanism for this reduction is PTEN-mediated eosinophil degranulation and suppression of interleukin (IL)-4 and IL-5. These findings indicate that use of PTEN may be a good therapeutic strategy for the management of allergic inflammation.

Key words: **PTEN • bronchial asthma • eosinophilia • phosphatidylinositol 3-kinase • signal transduction • inflammation**

Abbreviations: **Ad** – adenovirus, **BAL** – bronchoalveolar lavage, **ECP** – eosinophil cationic protein, **PI3-kinase** – phosphatidylinositol 3-kinase, **PIP3** – phosphatidylinositol 3,4,5-triphosphate, **PTEN** – phosphatase and tensin homologue deleted on chromosome 10.

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INTRODUCTION

Asthma is a common disorder of the airways in which they contract too much and too easily. The burden of asthma appears to be increasing worldwide, especially in societies undergoing rapid urbanization, and both morbidity and mortality from asthma have increased in many parts of the world, making it a global health concern. The Global Strategy for Asthma Management and Prevention Report¹⁷ stated that the definition of asthma is based on the functional consequences of airway inflammation, i.e. "Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation causes an associated increase in airway responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough, particularly at night and/or early morning. These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment." The treatment of asthma is internationally agreed upon and guidelines have been developed for the management of it¹⁷. The treatment of asthma is directed against airway obstruction and inflammation. Asthma treatment has two components. The first is the use of acute reliever agents (i.e. bronchodilators) for acute asthmatic airway obstruction. The second is the use of controller treatments, which modify the asthmatic airway environment. Of controller treatments, corticosteroids are the most potent anti-inflammatory drugs for use in the treatment of asthma. However, there is still clinically important subset of asthmatics who may not respond favorably to steroids.

Eosinophils are one of the most important cells of airway inflammation. Eosinophil accumulation and subsequent activation in bronchial tissues play critical roles in the pathophysiology of bronchial asthma⁵. Eosinophils are deleterious in asthma by the release of toxic granule proteins, including major basic protein, eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin, oxygen free radicals, eicosanoids, T helper 2-like cytokines, and growth factors. Once activated, products from eosinophils contract bronchial smooth muscles, increase vascular permeability, and induce airway hyperresponsiveness. Many inflammatory mediators attract and activate eosinophils via signal transduction pathways involving the enzyme phosphatidylinositol 3-kinase (PI3-kinase). Several studies using wortmannin, a specific inhibitor of PI3-kinase, have revealed the involvement of PI3-kinase in the biochemical transduction of activation signals generated by many inflammatory mediators in eosinophils^{3, 4, 13, 19}.

Wortmannin plays a role in preventing the development of airway hyperresponsiveness, either by preventing the eosinophil infiltration of bronchial tissues or their activation on arrival.

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), also known as MMAC-1 (mutated in multiple advanced cancers) or TEP-1 (TGF- β -regulated and epithelial cell-enriched phosphatase), was discovered in 1997 as a new tumor suppressor. It is now known to play major roles not only in suppressing cancer, but also in embryonic development, cell migration, and apoptosis¹⁸. PTEN functions primarily as a lipid phosphatase to regulate crucial signal transduction pathways¹⁸. PTEN has been implicated in regulating cell survival signaling through the PI3-kinase/Akt pathway. PTEN opposes the action of PI3-kinase by dephosphorylating the signal lipid phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3, produced by PI3-kinase following activation by receptor tyrosine kinases, activated Ras or G proteins, leads to the stimulation of several downstream targets, including the serine/threonine protein kinase Akt (also known as protein kinase B), which affects a variety of important cell functions¹.

Recently we demonstrated that administration of an adenovirus (Ad) gene transfer vector expressing a PTEN cDNA or PI3-kinase inhibitors reduced the inflammation and airway hyper-responsiveness in a murine model of allergic asthma, and the inhibition of PI3-kinase may be a good therapeutic strategy⁷.

This review briefly discusses the structure and functions of PTEN, but focuses primarily on the role of PTEN or PI3-kinase inhibitors on the airway inflammation of bronchial asthma.

STRUCTURE OF PTEN

Analysis of the structure of PTEN has identified two major domains^{8, 16}. The carboxyl (C)-terminal domain (amino acids 186-403) contains a lipid-binding C2 domain (amino acids 186-351), PEST domains (amino acids 350-375 and 379-396), and a PDZ domain (Fig. 1). The C2 domain appears to bind PTEN to the plasma membrane, and it might orient the catalytic domain appropriately for interactions with PIP3 and other potential substrates. The PEST domains are critical for PTEN stability. Mutagenesis studies demonstrate that phosphorylation of certain serine and threonine residues (S380, T382, and T383) can modulate both the enzymatic activity and the stability of PTEN. A key enzyme regulating the phosphorylation of this C-terminal cluster of serine and threonine residues appears to be the protein kinase

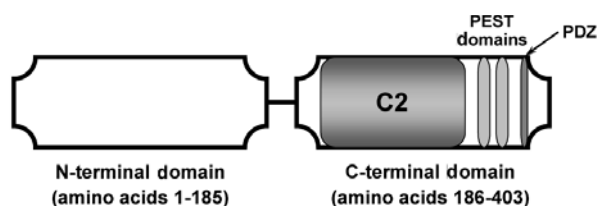


Figure 1. Schematic representation of protein domains of PTEN. Analysis of the structure of PTEN has identified two major domains: a carboxyl (C)-terminal domain (amino acids 186–403) and an amino (N)-terminal phosphatase domain (amino acids 1–185). The C-terminal domain contains the lipid-binding C2 domain (amino acids 186–351), PEST domains (amino acids 350–375 and 379–396), and a PDZ domain.

CK2, which modulates PTEN stability to proteasome-mediated degradation. The PDZ domain is important in protein-protein interactions. A PDZ domain in the tail might also play roles in altering the balance of PTEN effects on potential downstream signaling targets, such as Akt, versus some other system, such as Rac signaling. The C-terminal domain is composed of antiparallel β -sheets that are linked together by short α -helices.

PTEN also contains an amino (N)-terminal phosphatase domain (amino acids 1–185) that has a structure resembling previously characterized protein tyrosine phosphatases and contains an enlarged active site that can account for its ability to bind PIP3. The majority of PTEN mutations occur within this domain. The N-terminal phosphatase domain is composed of β -sheets surrounded by α -helices.

FUNCTION OF PTEN

PTEN is a recently identified tumor suppressor and is inactivated in a variety of cancers, such as glioblastoma, endometrial carcinoma, prostate carcinoma, melanoma, and small-cell lung cancer. Germ-line mutations of PTEN are the cause of Cowden syndrome, which is characterized by multiple hamartomas and an increased risk for development of tumors in a variety of tissues, and Bannayan-Riley-Ruvalcaba syndrome, which is characterized by lipomatosis, hemangiomas, macrocephaly, and speckled penis¹⁶. PTEN is also essential for embryonic development¹⁸. All PTEN-knockout mice die before birth, demonstrating a requirement of PTEN in embryogenesis. PTEN is a dual-specificity phosphatase that dephosphorylates protein substrates as well as lipid substrates. PTEN has been implicated in regulating cell survival signaling through the PI3-kinase/Akt pathway^{1, 11, 12, 14, 18} (Fig. 2). PTEN dephosphorylates the D3 position of the key lipid second messenger PIP3. In addition, PTEN has weak protein tyrosine

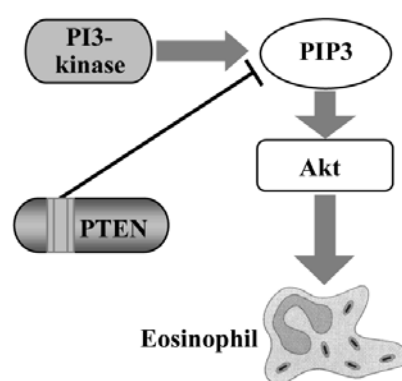


Figure 2. Schematic diagram of PTEN signaling in asthma. PTEN opposes the action of PI3-kinase by dephosphorylating the signal lipid PIP3. PIP3, produced by PI3-kinase following activation by receptor tyrosine kinases, activated Ras or G proteins, leads to the stimulation of several downstream targets, including the serine/threonine protein kinase Akt, and thereby affects eosinophil activation.

phosphatase activity, which may target focal adhesion kinase and/or Shc and thereby modulate other complex pathways. However, the major function of PTEN appears to be down-regulation of the PI3-kinase product PIP3. Physiologic roles of PTEN have been characterized in the regulation of many normal cell processes, including cell growth, adhesion, migration, invasion, and apoptosis^{2, 10, 18}. Recent studies revealed other functional consequences of PTEN action, such as the effect on the regulation of angiogenesis, non-insulin-dependent diabetes, and inflammation^{7, 9, 16}.

PTEN OR PI3-KINASE INHIBITORS IN ASTHMA

Bronchial asthma is an inflammatory disease of the airways characterized by airway obstruction and increased airway responsiveness. Eosinophils play important roles in the pathophysiology of asthma⁵. Although considerable controversy remains regarding the relationship between bronchial eosinophilic inflammation and airway hyperresponsiveness, it is believed that the eosinophils degranulate to release toxic granule proteins⁶ and that these products can cause airway hyperresponsiveness. Many inflammatory mediators attract and activate eosinophils via signal transduction pathways involving the enzyme PI3-kinase^{3, 4, 13, 15, 19}. Recently we have demonstrated that PI3-kinase activity was increased significantly after allergen challenge in a murine model of asthma⁷. Administration of PI3-kinase inhibitors, i.e. wortmannin or LY294002, reduces the airway inflammation. We have also found that the increased ECP levels in the bronchoalveolar lavage (BAL) fluids after allergen inhalation are significantly reduced by the administration of wortmannin or LY-294002. In addition, the

increased interleukin (IL)-4 and IL-5 levels in BAL fluids after allergen inhalation are significantly reduced by the administration of wortmannin or LY-294002. Our data are consistent with the results obtained in the study reported by Tigani et al.¹⁵ that wortmannin inhibits the increased number of eosinophils and eosinophil peroxidase activity recovered from the BAL fluid of allergen-challenged animals. Palframan et al.¹³ reported that wortmannin inhibited IL-5-induced selective release of eosinophils from perfused bone marrow, as well as selective eosinophil chemokinesis *in vitro*. The use of PI3-kinase inhibitors have revealed the involvement of PI3-kinase in the transduction of activation signals generated by many inflammatory mediators in eosinophils. These findings demonstrate that PI3-kinase plays a key role in the induction and maintenance of asthma.

PTEN protein expression and PTEN activity were decreased in allergen-induced asthma. Immunoreactive PTEN localized in the epithelial layers around the bronchioles of control mice, which dramatically disappeared in allergen-induced asthmatic lungs. The epithelial layer is one of the sites of the effect since this is the site of reduced PTEN expression and rescued expression with administration of AdPTEN. These results suggest that the epithelia, once insulted, may elicit the attraction of eosinophils and that this recruitment is impeded with inhibitors of PI3-kinase. Immunocytologic analysis of BAL fluids showed localization of immunoreactive PTEN in BAL cells from the control mice. However, immunoreactive PTEN was markedly reduced in precipitated cells, especially eosinophils, from allergen-exposed mice. Administration of AdPTEN recovered the immunoreactive PTEN expression in BAL eosinophils. The increased ECP levels in the BAL fluids after allergen inhalation were significantly reduced by the administration of PTEN. Others have shown that PI3-kinase inhibitors are potent inhibitors of eosinophil degranulation^{3, 4, 13, 15, 19}. PI3-kinase inhibitor potentially inhibits eosinophil peroxidase release from eosinophils⁴, and blocks neuro-peptide-induced chemotaxis and IL-5-induced β_2 -integrin-dependent adhesion to intercellular adhe-

sion molecule type 1-coated surfaces in human eosinophils^{3, 19}. Based on the effectiveness of PI3-kinase inhibitors and the decreased expression of PTEN in asthmatic mice, we administered AdPTEN to examine its effect. PTEN was effective in reducing all signs of asthma examined. The administration of PTEN, which opposes the action of PI3-kinase, might reduce eosinophilic inflammation and airway hyperresponsiveness in allergen-challenged mice, probably by inhibiting eosinophil degranulation and suppression of IL-4 and IL-5 concentrations in the airway. These findings clearly indicate that alterations in PTEN and PI3-kinase levels are implicated in the pathogenesis of bronchial asthma.

CONCLUSIONS

Asthma is an extremely common disorder affecting men and women equally. The worldwide prevalence of asthma has increased more than 30% since the late 1970s. Most of these patients have mild-to-moderate disease that can be controlled with appropriate treatment. However, it is estimated that 5–10% of patients with asthma have severe disease that is unresponsive to typical therapeutics, including corticosteroids. Therefore, new therapeutic approaches are needed to gain insights into a difficult therapeutic and possibly novel mechanistic area of asthma.

Several studies using PTEN and PI3-kinase inhibitors have revealed the involvement of PI3-kinase in the biochemical transduction of activation signals generated by many inflammatory mediators in eosinophils (Fig. 2). PTEN and PI3-kinase inhibitors play a role in preventing the development of airway inflammation, either by preventing eosinophil infiltration of the bronchial tissues or their activation on arrival. Therefore, PTEN may be a good therapeutic strategy for the regulation of allergic inflammation. While we expand our knowledge of the regulation of signal transduction such as PTEN or PI3-kinase inhibitors, we can learn the pathophysiology of bronchial asthma and design a selective therapy to function like a “magic bullet” against bronchial asthma.

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