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Role of breast regression protein-39/YKL-40 in asthma and allergic responses

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BRP-39 and its human homolog YKL-40 have been regarded as a prototype of chitinase-like proteins (CLP) in mammals. Exaggerated levels of YKL-40 protein and/or mRNA have been noted in a number of diseases characterized by inflammation, tissue remodeling, and aberrant cell growth. Asthma is an inflammatory disease characterized by airway hyperresponsiveness and airway remodeling. Recently, the novel regulatory role of BRP-39/YKL-40 in the pathogenesis of asthma has been demonstrated both in human studies and allergic animal models. The levels of YKL-40 are increased in the circulation and lungs from asthmatics where they correlate with disease severity, and CHI3L1 polymorphisms correlate with serum YKL-40 levels, asthma and abnormal lung function. Animal studies using BRP-39 null mutant mice demonstrated that BRP-39 was required for optimal allergen sensitization and Th2 inflammation. These studies suggest the potential use of BRP-39 as a biomarker as well as a therapeutic target for asthma and other allergic diseases. Here, we present an overview of chitin/chitinase biology and summarize recent findings on the role of BRP-39 in the pathogenesis of asthma and allergic responses.

Key Words: BRP-39; human CHI3L1 protein; asthma; hypersensitivity

INTRODUCTION

BRP-39 was discovered in mouse breast cancer cells.[1] Subsequently, a variety of homologues with different names were described including human HcGP-39, human YKL-40, porcine 38 kDa heparin-binding glycoprotein (GP38K), bovine 39 kDa whey protein and drosophila Imaginal Disc Growth Factors.[2-5] BRP-39 and YKL-40 are on chromosomes 1 and 2 in mouse and human, respectively, and are synthesized as 39 kDa proteins that lack chitinase activity. A variety of lines of evidence in a variety of species and modeling systems have implicated BRP-39-like molecules in the pathogenesis of tissue remodeling. In the breast, the expression of BRP-39 homologue, bovine 39 kDa whey protein, are increased during the involution phase after the cessation of lactation where they are felt to play an important role in the ongoing extensive glandular remodeling.[2] In drosophila, BRP-39-like and YKL-40-like molecules that lack chitinase activity have been shown to be growth factors[5] and in porcine systems, GP38K induces the differentiation of cultured vascular smooth muscle cells.[4] Human YKL-40 is also produced by cultured chondrocytes and synovial cells where it regulates cell proliferation and survival[3] and has mitogenic effects on human skin and lung fibroblasts and synoviocytes.[6] Interestingly, YKL-40 may also play an important role in disease pathogenesis because circulating levels of this moiety are elevated in a variety of diseases including metastatic breast cancer, hepatic fibrosis, severe purulent meningitis and community acquired pneumonia.[7-10] Increased levels of YKL-40 have also been noted in rheumatoid arthritis, atherosclerosis and osteoarthritis where they correlate with disease activity.[11-13] Recently, studies from our laboratory and others demonstrated that the levels of YKL-40 were increased in the circulation and lungs from asthmatics where they correlated with disease severity and CHI3L1 polymorphisms correlated with serum YKL-40 levels, asthma and abnormal lung function.[14-16] An additional promoter variant of CHI3L1 has been identified to be associated with atopic phenotypes in children.[17] In addition, recent studies using BRP-39 null mutant mice demonstrated blunted Th2 inflammatory responses when subjected to ovalbumin sensitization and challenge, further highlighting an important in vivo role of BRP-39 in the pathogenesis asthma or other allergic diseases.[18] This re-

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view briefly overviews the general biology of the chitin/chitinases and then focuses on the biological role of BRP-39/YKL-40 in the pathogenesis of asthma and allergic responses.

BIOLOGY OF CHITIN, CHITNASES, AND CHITNASE-LIKE PROTEINS

Chitin and chitinases

Chitin is a polymer of N-acetylglucosamine which has no mammalian counterpart. Following the cellulose in wood and paper, chitin is the second most abundant polysaccharide in nature. It is an essential component of fungal cell walls, the exoskeletons of crabs, shrimp and insects, the microfilarial sheath of nematodes and the digestive tracts of many insects.[19-26] These pathogens use chitin in a number of ways in their life cycles. Most commonly, chitin protects the pathogen from the harsh conditions inside the animal or plant host or in its environment.[27] Thus, an absence of chitin can lead to the death of the pathogen. Chitin deposition is regulated by biosynthesis and degradation. Chitinases, which are endo-b-1,4-N-acetylglucosamidases, are key degrading enzymes that have been studied most intensely in lower life forms. They are produced in significant quantities by hosts defending against infections with chitin-containing organisms. This attempt to damage the chitin coat of the infecting organism is part of the innate immune response against a chitin-containing pathogen.[19] It also produces differentially sized chitin fragments which can trigger innate immunity pattern recognition receptors to induce IL-17, TNF and/or IL-10 elaboration.[19,28,29] Chitinases also contribute to the life cycle of chitin-containing fungi and parasites where they control growth and molting. They are also used by pathogens to invade or exploit chitin-containing structures in the host. This allows them to establish successful infections and thus play a critical role(s) in the transmission of infection from one vertebrate host to another by insect vectors.[26,27,30] As a result of the importance of chitin in the protection of pathogens and the importance of appropriately regulated chitinase production in the life cycle of pathogens, chitin synthesis inhibitors and chitinase inhibitors have received significant attention as potential biopesticides to eradicate insects, fungi and helminthic parasites.[31,32]

Mammalian chitinase and chitinase-like genes

Until recently it was assumed that mammals lacked chitinases. Recent studies in humans and rodents, however, have identified a family of chitinases and CLP in both species referred to as the 18 glycosyl hydrolase family. Acidic mammalian chitinase (AMCase), chitotriosidase, oviductin, YKL-40 and YKL-39 have been described in humans, while YM-1, YM-2, AMCase, oviductin, and BRP-39 have been described in mice.[20,21,33-35] Recent studies from our laboratory have also described mouse chitotriosidase.[36] YM-1 and YM-2 may be mouse-specific because comparable genes have yet to be described in man. They are produced by macrophages after parasitic[34] or fungal infection.[37] AMCase is produced by epithelial cells, macrophages and eosinophils at sites of Th2 inflammation.[38] Interestingly, IL-13 is necessary and sufficient for the induction of this chitinase.[38] In all cases, these moieties have a moderate degree of sequence homology with lower life form chitinases. However, in contrast to the chitinases in lower life forms, only chitotriosidase and AMCase have true chitin-degrading activity.[20] Because of mutations in their highly conserved putative enzyme sites, BRP-39, YKL-40 and the other CLP do not have chitinase activity.[33,34] As a result, their roles in biology are particularly enigmatic. A complete understanding of the biology of the chitinases and CLP requires elucidation of the roles of true chitinases and the chitinase-like proteins. Insights into the roles of AMCase have been obtained from studies in our laboratory[38] and others.[39] Recent studies using transgenic and null mutant mice shed on light on the biologic properties of BRP-39 and YKL-40, the murine and human versions of this prototypic CLP in the development of allergic responses and tissue remodeling.[18]

Functions of mammalian chitinase-like proteins

One of the most pressing issues in chitinase biology relates to our almost complete lack of understanding of the functions of these strongly conserved (and therefore presumably biologically important) moieties in mammals and man.[33] Mammalian CLP are induced at sites of inflammation (such as parasitic infections)[34] and remodeling.[9] This raises the possibility that these molecules play active roles in human anti-parasite and anti-infective defense and repair responses. In accord with this concept, microarray analysis has demonstrated that the genes encoding chitinases are among the most prominently induced genes in parasite-challenged[40] or IL-13-challenged lung tissue.[41] It is important to point out, however, that the majority of the mammalian chitinase-like molecules do not have true chitinase activity (only chitotriosidase and AMCase have chitinolytic activity). Thus, the biologic roles of these molecules are even less adequately understood. It is reasonable to believe, however, that mammalian enzymes with true chitinase activity (such as AMCase and chitotriosidase) can play a direct role in host responses to chitin-containing pathogens.[39] It is also reasonable to postulate that chitinase-like proteins such as BRP-39/YKL-40 can also: (a) play a role as sentinels that trigger responses to parasites, infections and/or antigen challenge; (b) attract eosinophils and T cells to sites of parasitic infection[34,35,42] and/or; (c) generate or modulate tissue inflammation, immunity and/or remodeling. Recently, with the development of BRP-39 null mutant mice and lung specific YKL-40 overexpressing transgenic mice, in vivo regulatory role of BRP-39/YKL-40 in allergic inflammation and tissue response have been described.[18] These studies demonstrated that BRP-39 is a key regulator of Th2 inflammation, M2 macrophage differentiation and Th2 cell and

macrophage apoptosis/cell death. These findings provide novel insights into the in vivo roles of BRP-39 in allergic sensitization process and effector function of Th2 cytokines. They represent a new level of understanding about the processes that regulate inflammatory cell survival and tissue remodeling responses, the pathologic hallmarks of asthma and allergic diseases.

Chitinase and chitinase-like proteins (C/CLP) in tissue remodeling

Recently, a number of studies suggest an important role of C/CLP in disease pathogenesis characterized by inflammation and pathologic tissue remodeling. The activity and levels of chitotriosidase in serum and BAL were higher in the patients with sarcoidosis, or with idiopathic pulmonary fibrosis, than in controls.[43,44] Several studies also suggested that CLP such as YKL-40 or mouse Ym-1 or Ym-2 could be involved in tissue remodeling processes. Serum YKL-40 was significantly related to the degree of liver fibrosis, and staining of YKL-40 antigen was higher in areas with fibrosis, particularly in areas with active fibrogenesis.[45,46] The animal models that accompany this tissue remodeling process also demonstrated significant changes in C/CLP expression at sites of inflammation or remodeling. Th2-inducing pathogens *Schistosoma mansoni* and *Nippostrongylus brasiliensis* cause granulomatous inflammation and liver fibrosis in the infested mice. In that model, AMCase and Ym-1 expression were significantly increased along with type 2 cytokines such as IL-13 and IL-4.[47] In the mice with pulmonary fibrosis induced by crystalline silica exposure,[48] or herpesvirus,[49] there are close associations between expression of C/CLP and the degree of tissue remodeling. In this regard, it is intriguing to speculate that C/CLP, such as AMCase or YKL-40, play an important role in tissue remodeling process in chronic asthmatic patients. However, it is still not clear whether C/CLP actively participate in the tissue remodeling process or indirectly modulate the process through regulation of other cytokines and/or growth factors. Further mechanistic studies using specific gene targeted animal models or transgenic models will be required to define more specific functions of C/CLP in tissue remodeling processes.

REGULATORY ROLE OF BRP-39/YKL-40 IN ASTHMA AND ALLERGIC RESPONSES

Role of BRP-39 in allergic inflammation and tissue remodeling

Recently, BRP-39 null mutant mice and lung-specific YKL-40 overexpressing transgenic mice have been generated and used to define the functional role of BRP-39 in allergic and Th2 cytokine effector functions.[18] These studies demonstrated that the null mutant mice have a significant defect in antigen-induced Th2 inflammation and IL-13-inuced inflammation and remodeling. These studies further demonstrated that BRP-39 and YKL-40 accomplish this, at least in part, by inhibiting inflammatory cell (T cell, macrophage and eosinophil) apoptosis/cell death while inhibiting Fas expression and stimulating protein kinase B/AKT phosphorylation. BRP-39 and YKL-40 were also shown to stimulate dendritic cell accumulation and activation, and to induce alternative macrophage activation. These studies suggest that BRP-39 may involve multiple stages of allergic responses by regulation of sensitization and Th2 cytokine effector functions. The defects in antigen sensitization and Th2 inflammation in BRP-39 null mutant mice can be explained by a marked decrease in the numbers of myeloid and plasmacytoid dendritic cells and the ability of these cells to be activated after antigen exposure. The hypothetical regulatory pathways of BRP-39 in allergic inflammation and tissue remodeling response has been illustrated in the Fig. 1. However, the exact regulatory mechanism of BRP-39 in dendritic cell function to drive Th2 polarization still remains to be determined. The specific role of BRP-39 in allergic response was further supported by the rescue experiment by generating BRP-39 null mice with epithelial cell-specific YKL-40 transgenic mice. In these mice, the epithelial YKL-40 totally rescues the deficient Th2 response in BRP-39 null animals, suggesting that secreted YKL-40 is an important soluble factor driving asthma-like Th2 inflammatory responses. These studies also identified a novel regulatory function of BRP-39 in IL-13-induced tissue fibrosis. Previous studies from our laboratory demonstrated that the fibrogenic effects of IL-13 are mediated, at least in part, by the ability of IL-13 to induce and activate TGF-β_1.[50] Intriguingly, TGF-β_1 induction of the lungs of IL-13 transgenic mice was significantly decreased in mice with a deficiency of BRP-39. It may explain the general regulatory role of BRP-39 in tissue remodeling in various diseases. However, the cellular and molecular mechanism of BRP-39 intervening IL-13-induced TGF-β_1 production and activation need to be further determined. Finally, BRP-39 has a potent regulatory role in cell death responses that may responsible for proinflammtory roles of BRP-39 in allergic inflammation and in other inflammatory diseases, at least in part. BRP-39 has been shown to inhibit Fas- or TNF-α-induced cellular apoptosis while enhancing PKB/Akt pathways in macrophages and T cells.[18] The properties of YKL-40 in activating MAP kinase and PKB/Akt pathways have been demonstrated in in vitro assays with connective tissue cells.[6] Inflammatory cell apoptosis has been regarded as a mechanism of resolution of inflammation.[51,52] Thus, further mechanistic evaluation on the regulatory role of BRP-39 in specific apoptosis pathways will be necessary to understand the in vivo function of BRP-39 in asthmatic inflammation and tissue remodeling.

YKL-40 as a biomarker and potential therapeutic target

A variety of inflammatory cells (e.g., neutrophils, macrophages and differentiating monocytes) as well as structural cells (e.g., differentiated smooth muscle cell, chondrocytes, synovial cells, endothelial cells, and tumor cells) endogenously

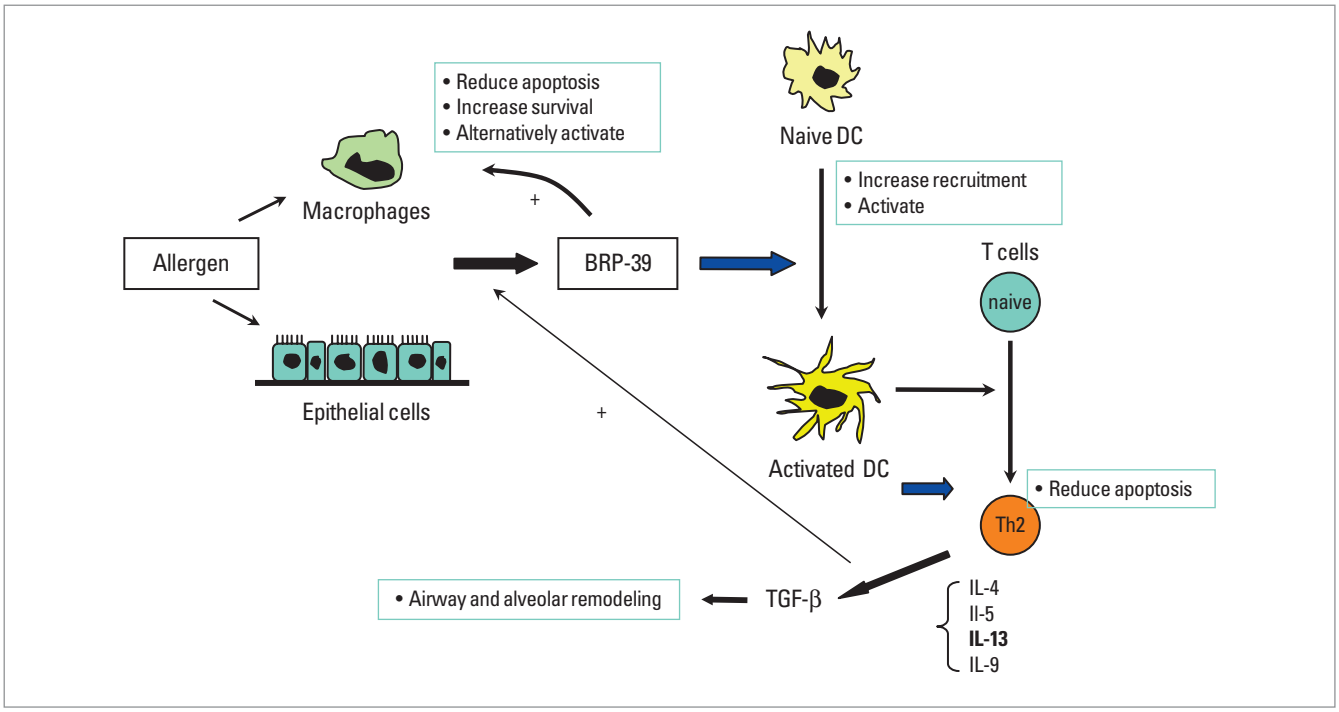

Fig. 1. The proposed regulatory pathway of BRP-39 in allergic inflammation and tissue remodeling. Macrophages and epithelial cells are the primary cells expressing BRP-39 in the lung after allergen sensitization and challenge. BRP-39 increases the dendritic cell numbers in the lung and further activates and leads to enhanced Th2 polarization. BRP-39 also increases Th2 cells by reduction of T cell apoptosis or increase of cell survival. TGF-β or other growth factors produced by Th2 cytokine stimulation leads to airway or alveolar remodeling. BRP-39 and Th2 cytokines such as IL-13 or IL-4 further contribute to the production of BRP-39 via regulation of cell death responses or alternative macrophage activation.

express YKL-40.[3,53-58] Intriguingly, increased levels of YKL-40 protein and/or mRNA have been noted in a variety of diseases characterized by inflammation, tissue remodeling, and aberrant cell growth. They include rheumatoid arthritis,[59] osteoarthritis,[60] giant cell arthritis,[61] sarcoidosis,[62] sclerosis,[63] diabetes,[64] atherosclerosis,[65,66] inflammatory bowel disease,[67] liver fibrosis[45,68] and several malignancies.[58,69,70] Recently, elevated levels of YKL-40 in the BAL and serum in smokers with COPD were reported.[71] These significant associations of YKL-40 with a variety of disease development or progression renders YKL-40 as a useful diagnostic or prognostic biomarker.[72,73] In addition, in many of these disorders the levels of YKL-40 reflect the activity and natural history of the disease.[13,14,74,75] This is nicely illustrated in studies from our laboratory and others which demonstrated that elevated levels of serum YKL-40 are seen in patients with asthma which correlate with the levels of lung tissue YKL-40 and disease severity.[14] These studies also highlighted polymorphisms in chitinase 3-like-1 that correlated with the levels of circulating YKL-40, the presence of asthma, and compromised lung function.[15] The potential importance of YKL-40 can also be seen in rheumatoid arthritis, coronary artery disease, solid cancers and death in the elderly where elevated serum YKL-40 levels correlate with the severity of joint involvement, the number of blocked coronary arteries, short disease free intervals, and all cause mortality, respectively.[13,74,76,77] As a result, YKL-40 is a prognostic biomarker and has been proposed to be a therapeutic target in conditions characterized by acute or chronic inflammation, extracellular matrix remodeling, fibrosis and cancer.[76-78] In this regard, recent animal studies demonstrating an essential role of BRP-39 in the pathogenesis of allergic inflammation and tissue remodeling,[18] legitimate the usefulness of BRP-39/YKL-40 as a therapeutic target for asthma and other allergic diseases.

UNSOLVED ISSUES AND FUTURE RESEARCH NEEDS

Mechanisms underlying BRP-39/YKL-40 effector responses

BRP-39/YKL-40 is a secreted protein that is synthesized with a propeptide that is removed to reveal the mature protein. X-ray crystal analysis has revealed a (beta/alpha) 8 barrel fold with a 43 AA carbohydrate binding cleft.[79] Despite this structural knowledge, the carbohydrate binding repertoire of BRP-39/YKL-40 has not been fully defined. Its ability to bind with high affinity to chitin has been noted above. Recently, it has been shown to bind to heparin and collagen with lower affinity. The roles of BRP-39/YKL-40 in inflammation, remodeling and angiogenesis and its ability to act as a mitogen, chemotactic factor and growth factor are believed to be the result of cell surface li-

gand binding events.[79] In accord with this concept, BRP-39/YKL-40 has been shown to activate mitogen activated protein kinase (MAPK), PI-3 kinase (PI3K) and PKB/Akt[79] signaling pathways. While the activation of these pathways is linked to ligand binding to a cell surface receptor, no cell surface BRP-39/YKL-40 binding proteins have been identified. In fact, a biologically active receptor for any C/CLP has not been identified. Thus, the identification of the ligand-receptor interactions that mediate the effector responses of BRP-39/YKL-40 and related moieties is one of the most pressing challenges in C/CLP biology.[79]

BRP-39 in allergic sensitization and allergic responses with chitin or chitin-containing allergen

Although it has been shown that BRP-39 is required for optimal sensitization with ovalbumin (non-chitin containing allergen),[18] the exact role of BRP-39 in sensitization processes is still largely undefined. How dose BRP-39/YKL-40 regulate dendritic cell functions or subsequent T cell polarization? What is the role of BRP-39/YKL-40 in allergic responses with chitin or chitin-containing allergens (e.g., house dust mite, pollen, Ragweed etc.) other than ovalbumin? Although some evidence suggests that BRP-39 plays a similar role with chitin-containing house dust mite challenge as with ovalbumin,[18] the regulatory role of BRP-39 in allergic responses with chitin or chitin-containing allergen has not been fully evaluated. Those are the remaining questions that need to be fully addressed in the future for a clearer understanding on the role of BRP-39/YKL-40 in asthma and other allergic responses.

Potential interaction of BRP-39 with AMCase or other C/CLP

Previous studies demonstrated that AMCase also play an important role in allergen-induced Th2 inflammation and effector function of IL-13.[38] Because these functional similarities, it raises the question regarding a potential redundancy of C/CLP in the regulation of allergic inflammation. Although there is overlap in the expression and regulatory pathways between these two molecules, many pieces of evidence suggest specific regulatory roles of BRP-39 that are distinctive from those of AMCase.[18] First, the regulation of the expression of BRP-39 and AMCase is not the same: IL-13 induces both AMCase and BRP-39, but BRP-39 was induced by IFN- while AMCase was not. Second, double immunohistochemisty demonstrated that sites of BRP-39 and AMCase differentially expressed, depending on the cells. BRP-39 staining was more pronounced in alveolar epithelial cells and macrophages, while AMCase had its abundance in airway epithelial cells. Lastly, the levels of AMCase were not significantly changed in IL-13 transgenic lungs with BRP-39 null mutation. Because IL-13-induced inflammatory and tissue phenotypes were drastically changed in the absence of BRP-39, we can speculate that BRP-39 is required for IL-13 effector functions independent of AMCase. However, we still do not have clear answers regarding whether these two molecules have close interaction in the regulation of allergic responses, partly because currently we do not have appropriate murine models such as AMCase null mutant mice or transgenic mice to evaluate specific function of AMCase in relation to BRP-39. In this regard, comprehensive in vivo and in vitro studies to define potential C/CLP interactions will be necessary to understand C/CLP regulation of allergic responses.

CONCLUSIONS

YKL-40, a human homolog of BRP-39, a chitinase-like protein, has been reported to be associated with a number of diseases characterized by inflammatory and tissue remodeling responses. However, the in vivo role of BRP-39/YKL-40 in the pathogenesis of specific diseases has been elusive until the recent development of gene-specific null mutant mice and overexpressing transgenic mice. Studies from our laboratory demonstrate that BRP-39 is stimulated by IL-13 and Th2 inflammation and that null mutations of BRP-39 diminish Th2 and IL-13-induced inflammation and remodeling.[18] They also demonstrate that BRP-39/YKL-40 inhibits T cell and macrophage apoptosis/cell death while inhibiting Fas expression, increasing the activation of PKB/Akt and inducing M2 macrophage differentiation.[18] When combined with the recent demonstration that the levels of YKL-40 are increased in the circulation and lungs from asthmatics where they correlate with disease severity[14] and that *CHI3L1* polymorphisms correlate with serum YKL-40 levels, asthma and abnormal lung function,[15] these studies further provide novel insight on the regulatory roles of BRP-39 in IL-13 and/or Th2-mediated inflammation and tissue responses. They also legitimate the usefulness of BRP-39/YKL-40 as a diagnostic biomarker as well as potential therapeutic target of asthma and other allergic inflammatory diseases. For better understanding of the effector function of BRP-39/YKL-40 in inflammation and tissue remodeling, more mechanistic studies directed to define the molecules that interact with these chitinase-like proteins such as receptor or signaling proteins, will be warranted in the future.

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