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Expression of galectin-9 by IFN-γ stimulated human nasal polyp fibroblasts through MAPK, PI3K, and JAK/STAT signaling pathways

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ABSTRACT

Galectin-9 exhibited potent and selective eosinophil chemoattractant activity and attracted eosinophils in vitro and in vivo. Nasal polyposis is a chronic inflammatory disease of the upper airway characterized by the marked presence of inflammatory cells, particularly eosinophils. Thus, galectin-9 may be implicated in the pathogenesis of nasal polyposis. The study was designed to investigate whether interferon-gamma (IFN-γ) can induce the augmentation of galectin-9 expression and induce the expression of galectin-9 in nasal polyps. We examined the correlation between galectin-9 expression and eosinophil infiltration in nasal polyps. In addition, we identified the signaling pathways involved in the elevation of galectin-9 expression in response to IFN-γ. Our data demonstrate that the involvement of mitogen-activated protein kinases (MAPKs), phosphatidylinositol 3 phosphate kinase (PI3K), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) may play important roles in the selective recruitment of eosinophils in nasal polyp tissues through the production of galectin-9. These findings suggest that galectin-9 expression is associated with eosinophil infiltration in polyps of patients with nasal polyposis.

1. Introduction

Nasal polyposis is a chronic inflammatory disease of the nasal and paranasal sinus mucosa, characterized by the presence of edematous masses in the nasal and paranasal cavities, leading to nasal obstruction, secretion, loss of smell, headache, and reduced general well-being, resulting in a socio-economic burden. Nasal polyposis is a multifactorial disease that often coexists with asthma and other conditions such as cystic fibrosis, primary ciliary dyskinesia, and aspirin intolerance [1–5]. Nasal polyps are the most common mass lesions in the nose. Although many hypotheses have been suggested, the pathogenesis of all nasal polyps remains unclear. Histologically, nasal polyps are covered with respiratory epithelium, large quantities of extracellular edema and a dense inflammatory cell infiltrate consisting of mast cells, lymphocytes, neutrophils, eosinophils, and plasma cells. In the majority of nasal polyps, eosinophils comprise over 60% of the cell population [5]. Because NPs are often surgically removed, they may become a readily available source of tissue eosinophils [5–7]. Therefore, accumulation of eosinophils is a key factor in the development of nasal polyposis.

In addition to increased inflammatory cell infiltration, there is increased expression and production of a variety of pro-inflammatory mediators, including cytokines and chemokines, in nasal polyps. These cytokines and chemokines can contribute to chronic eosinophilic inflammation by regulating the migration, survival and activation of eosinophils. In recent years, eosinophil

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chemoattractants, such as eotaxin and regulated on activation normal T expressed and secreted (RANTES), have been detected in nasal polyps [3].

Galectins are an animal lectins family that have an affinity for β-galactosides and have a highly conserved sequence element in the carbohydrate-binding site [8,9]. At least 15 members have been identified in mammalian species. Each member of the family has a diversity of cellular functions including cell adhesion, proliferation, development, morphogenesis, tumor cell metastasis and immune regulation/innate immunity [10–16]. Among these, galectin-9, composed of 323 amino acids, has been isolated as a potent chemoattractant for eosinophils from activated T cells [17–19]. Galectin-9 exhibits potent eosinophil-chemoattractant activity that is as high as that of eotaxin and attracts eosinophils in vitro and in vivo [17]. Its expression has recently been evidenced in nasal polyps [20], where it may play an important role in recruiting eosinophils. These data suggest that galectin-9 plays a key role in nasal polyposis.

Nasal polyp fibroblasts are found in the stroma of nasal polyps and are the major cellular component of the polyp architecture. Nasal fibroblasts are important modulators of the local inflammation due to their capacity to release a variety of biologically active factors. Galectin-9 secreted by fibroblasts may play an important role in the activation and survival of eosinophils in local tissues [20]. However, there is no information about the mechanism of galectin-9 expression in human nasal polyp fibroblasts. In the present study, we examined the correlation between galectin-9 expression and eosinophil infiltration in nasal polyps. We also investigate the mechanism of galectin-9 expression in nasal polyp fibroblasts stimulated with IFN-γ.

2. Materials and methods

2.1. Grouping of tissue specimens

Nasal polyps were divided into two groups based on predominant type of infiltrating cells [21]. Polyps showing predominant infiltration of eosinophils were defined as the eosinophil-rich nasal polyps (ENPs), whereas the other types that did not show significant eosinophilia were defined as non-eosinophilic nasal polyps (NENPs).

2.2. Cell source and culture

Nasal polyps were obtained from the region of the middle meatus at the beginning of the surgical procedure. The patients had no history of nasal allergy, asthma, or aspirin sensitivity. The fibroblasts were grown in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% FBS (Sigma) and 1% antibiotics/antimycotics (Gibco-BRL). For each experiment, the fourth and eighth passages of the nasal fibroblasts were used at about 70% confluency.

2.3. Histological examination

Hematoxylin and eosin staining was performed to examine the infiltration of eosinophils. Nasal polyps were removed and fixed in 10% neutral buffered formalin. The specimens were then dehydrated and embedded in paraffin. For histological examination, 5 μm sections of the fixed embedded tissues were cut on a Leica model 2165 rotary microtome (Leica, Nussloch, Germany).

2.4. Immunohistochemistry

The nasal polyps were prepared to a thickness of 5 μm from formalin-fixed paraffin-embedded tissue and mounted on poly-L-lysine coated slides and then deparaffinized and rehydrated through a series of xylene and diluted alcohols. After being rinsed with distilled water, the sections were immersed in Tris/EDTA buffer (pH 9.0; Dako, Carpinteria, CA), incubated for 5 min at 120 °C (12–15 psi) in a microwave and were then allowed to cool for 20 min for antigen retrieval. Endogenous peroxidase activity was blocked by incubation with 3 percent H_2O_2 for 10 min at room temperature, and the slides were then washed in phosphate-buffered saline and immersed in Tris-buffered saline (TBS) buffer. Sections were incubated overnight with galectin-9 antibody (RD Systems, Minneapolis, MN) at 4 °C. The slides were then incubated with biotin-conjugated secondary anti-goat antibody and streptavidin-conjugated peroxidase. DAB was used as a chromogen, and Mayer's hematoxylin was used for counterstaining.

2.5. Reverse transcriptase-polymerase chain reaction

The total RNA was isolated using TRIzol reagent (Invitrogen, CA). The total RNA (1.0 μg) obtained from the cells was reverse-transcribed using M-MLV reverse transcriptase (Promega, Madison, WI) to produce the cDNA. RT-generated cDNA encoding the *galectin-9* gene was amplified using PCR. PCR was performed using selective primers for mouse *galectin-9* (5′-ATGTCCGAAGCAAACATCAC-3′ and 5′-TAATGTCCAGGAAGTAGGTG-3′). After amplification, portions of the PCR reactions were electrophoresed on an agarose gel.

2.6. Western blot analysis

Cells were washed three times with PBS and lysed in lysis buffer (1% Triton X-100, 1% deoxycholate, 0.1% NaN_3) containing a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Equal amounts of protein were separated on 10% SDS-polyacrylamide minigels. Proteins were transferred to Immobilon PVDF membranes (Millipore), and then, the membranes were blocked in 5% bovine serum albumin (BSA) and Tris-buffered saline-Tween (TBST, 100 mM Tris, pH 8.0, 150 mM NaCl and 0.1% Tween 20) for 1 h at room temperature. A specific antibody against galectin-9 (1:1000 dilution; RD Systems) was diluted in 5% BSA-TBST. After incubation with the appropriate primary antibody, membranes were incubated for 1 h at room temperature with a secondary antibody conjugated to horseradish peroxidase (1:10000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA). Following three washes in TBST, immunoreactive bands were visualized using the ECL detection system (Pierce, Rockford, IL). In a parallel experiment, nuclear protein was prepared using nuclear extraction reagents (Pierce) according to the manufacturer's protocol.

2.7. Eosinophil chemotactic activity

Eosinophil chemotactic activity was assessed in 24-well chambers (Costar, Cambridge, MA) containing a polyvinyl–pyrrolidone-free membrane with a pore size of 3 μm. Nasal polyp fibroblasts were pretreated with various kinase inhibitors for 30 min and were then stimulated with IFN-γ (100 U/ml) for 48 h. The culture media were harvested for analysis. The lower chamber was filled with 500 μl of culture media. An EoL-1 cell suspension (50 μl, 1×10^7 cells/ml) in DMEM supplemented with 10% FBS was placed in the upper chamber. The chemotaxis chambers were incubated in humidified air in 5% CO_2 at 37 °C for 2 h. After the polycarbonate filter was removed, cells adhering to upper surface were wiped away from the membrane. The membrane was fixed and stained with Diff-Quick (Merck, Germany) and mounted on a glass slide. Stained cells that had completely migrated through the filter were counted by means of light microscopy by counting 7 different