Inflammation-mediated upregulation of centrosomal protein 110, a negative modulator of ciliogenesis, in patients with chronic rhinosinusitis

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Background: Sinonasal mucosa in patients with chronic rhinosinusitis (CRS) is often devoid of motile cilia. This defect is presumed to result from prolonged inflammation, infection, or both. However, the mechanism underlying this observation is unknown. Recently, centrosomal protein 110 (Cp110) was shown to prevent the terminal step in ciliary maturation (ie, elongation), suggesting that Cp110 might be involved in pathological states in which ciliation is abnormal.

Objectives: First, we sought to investigate the expression of Cp110 in sinonasal mucosa from patients with CRS and control subjects. Second, we sought to determine the extent that inflammatory cytokines modulate Cp110 expression and ciliary maturation *in vitro*.

Methods: Sinonasal mucosal specimens from patients with and without CRS were analyzed for Cp110 mRNA and protein expression. Furthermore, human and murine nasal respiratory epithelial cultures were used to investigate Cp110 expression under normal growth conditions and in the presence of exogenous proinflammatory cytokines.

Results: Increased Cp110 mRNA and protein expression was found in sinonasal mucosal specimens from patients with CRS compared with that seen in control specimens. During ciliogenesis *in vitro*, the expression of Cp110 gradually decreased in cultures derived from patients without CRS but remained increased in cultures derived from patients with CRS. Furthermore, cultures grown in the presence of proinflammatory cytokines demonstrated increased levels of Cp110 expression with concomitant inhibition of ciliogenesis.

Conclusion: Increased Cp110 expression in mucosa from patients with CRS might contribute to the poor ciliation observed in patients with CRS. Exogenous cytokine exposure maintains increased levels of Cp110 expression. Regulation of Cp110 expression by inflammation warrants additional investigation because it might offer a novel target in the management of respiratory tract diseases. (J Allergy Clin Immunol 2011;128:1207-15.)

Key words: *Chronic rhinosinusitis, ciliated epithelial cell, ciliogenesis, cytokine, inflammation, centrosomal protein 110*

The predominant cell of the sinonasal epithelium is the ciliated cell, which plays a critical role in protecting the upper airway from inhaled pathogens and debris. Coordinated beating of cilia lining the mucosal surface of epithelial cells propels debris-laden mucus to the pharynx for elimination by the gastrointestinal tract.[1,2] When this mechanism fails, disease ensues because of mucosal stasis.

Multiple reports have demonstrated that sinonasal mucosa from patients with chronic rhinosinusitis (CRS) with and without nasal polyposis have significant morphologic changes compared with control mucosa, such as an abundance of nonciliated cells and ciliated cells with short or abnormal cilia.[3-6] To date, a mechanistic relationship between the primary inflammatory disease process in patients with CRS and poor ciliation has not been established. Moreover, it is unclear whether poor ciliation is due to decreased ciliation, increased cilia loss, or some combination. We set out to determine whether dysregulated ciliogenesis in the setting of CRS might contribute to the poor ciliation observed in patients with CRS.

Current understanding divides ciliogenesis into 4 steps: (1) multiplication of the centrioles, (2) migration of the centrioles to the apical surface, (3) formation of the basal body–associated structures, and (4) elongation of the cilia. Since the identification of the centrosome at the end of the 19th century by Boveri,[7] the majority of investigations into this organelle focused on its function in the cell cycle and development. The centrosome or microtubule-organizing centers (MTOCs) in eukaryotic cells is comprised of 2 centrioles and a surrounding electron-dense matrix, the pericentriolar material. The MTOC regulates cell motility, adhesion, and polarity in the interphase and facilitates the organization of the spindle poles during mitosis.[8] In addition, the centrioles have another distinct function in ciliated epithelial cells as the building blocks for basal bodies that initiate the growth of cilia.[9] Recently, it was discovered that downregulation of centrosomal protein 110 (Cp110) had a marked effect on centrosome duplication.[10-15] Chen et al[11] reported that deleting Cp110 in a cell line induced cytokinesis and centrosome separation with duplication defects and genomic instability. Then, by means of transmission electron microscopy, Cp110 was found to localize to the distal end of centrioles near the transition

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Abbreviations used
ALI: Air-liquid interface
Cp110: Centrosomal protein 110
CRS: Chronic rhinosinusitis
hCp110: Human centrosomal protein 110
HSEC: Human sinonasal epithelial cell
KC: Keratinocyte-derived chemokine
mCp110: Murine centrosomal protein 110
MSEC: Murine septum epithelial cell
MTOC: Microtubule-organizing center
SEM: Scanning electron microscopy

zone and to limit axoneme nucleation.[12] Interacting with centrosomal protein of 97 kd (Cep97), the complex is critical for normal cilia formation because deletion of either Cep97 or Cp110 results in aberrant cilia formation in proliferating cells,[16] whereas expression of Cp110 suppresses cilia formation in quiescent cells interacting with centrosomal protein of 290 kd (Cep290).[14] This negative regulation suggests that Cp110 expression suppresses cilia formation by blocking centriole duplication and preserving the parental centrioles to participate in centrosomal function.[13,15] In this series of studies, we first evaluated Cp110 expression in control mucosa and mucosa from patients with CRS, as well as the temporal expression of Cp110 protein during respiratory epithelial cell differentiation. Because inflammation plays a critical role in the establishment and progression of CRS, we next investigated the effect of selected proinflammatory cytokines that are associated with CRS on both human and murine sinonasal epithelial cell expression of Cp110 and ciliogenesis.

METHODS

Human tissue procurement

Patients were recruited from the Division of Rhinology of the Department of Otorhinolaryngology–Head and Neck Surgery at the University of Pennsylvania. Approval for the study was obtained from the institutional review board. Informed consent was obtained during the preoperative clinic visit or in the preoperative waiting room. The selection criterion for recruitment was a patient undergoing sinonasal surgery. Exclusion criteria included a history of systemic granulomatous disease, active smoking, or current use of corticosteroids (oral or topical) or antibiotics for 2 weeks before surgery. Patients with CRS met the objective and subjective guidelines for CRS outlined by the clinical practice guideline for adult sinusitis set forth by the Academy of Otolaryngology–Head and Neck Surgery.[17] Patients with CRS were subcategorized based on the endoscopic visualization of sinonasal polyposis. Twelve patients with CRS without nasal polyps and 17 patients with CRS with nasal polyps were included in the study. Control subjects ($n = 8$) had no history of CRS and no evidence of sinonasal mucosal inflammation on endoscopy but were undergoing sinonasal surgery for other indications, such as pituitary tumors, encephaloceles, or repair of cerebrospinal fluid rhinorrhea. Human sinonasal samples were used for 3 different analyses: quantitative real-time RT-PCR, Western blot analysis, and establishment of primary air-liquid interface (ALI) cultures. Mucosa for these studies was removed from the ethmoid sinuses posterior to the ethmoid bulla, thus avoiding any trauma caused by pledgets used at the beginning of surgery for decongestion. Because of the limited source material in patients without polyps, Western blot analysis and primary cultures (see below) were only established from patients with polyps and select control subjects who had extensive mucosal removal for surgical access.

ALI cultures

Murine nasal septal mucosa was harvested from 9-week-old C57BL/6J mice. All animal handling and manipulations were preapproved by the Animal Care Committee at the University of Pennsylvania Health Center, as well as the Philadelphia Veterans Affairs Medical Center. Detailed description of primary sinonasal epithelial cultures can be found in the Methods section in this article's Online Repository at www.jacionline.org or in previously published reports.[18,19] It is important to note that first-passage cultures were used for all these studies. Prior work by others demonstrated that these cultures are nearly 100% epithelial.[20] On days 0, 7, and 14 after conversion from submersion to ALI, both murine septum epithelial cell (MSEC) and human sinonasal epithelial cell (HSEC) ALI membranes were collected for quantitative real-time RT-PCR, Western blot analysis, and immunofluorescent staining.

RNA extraction

Total RNA was extracted from human sinonasal tissue or ALI cultures by using the RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Purity of the total RNA extracted was determined as the A_{260}/A_{280} ratio, with expected values between 1.8 and 2.0. cDNA was synthesized from 1 μg of DNase I–treated (Gibco, Gaithersburg, Md) total RNA, as described previously.[21]

Real-time RT-PCR

Quantitative real-time RT-PCR was performed on a Prism 7000 sequence detection system (Applied Biosystems, Foster City, Calif). The β-actin gene was used as a reference for sample normalization. For detection of Cp110, PCR was performed with the SYBR Green PCR kit (Applied Biosystems). Specific primer sequences can be found in the Methods section in this article's Online Repository. The relative fold increase of human Cp110 (hCp110) or murine Cp110 (mCp110) expression was calculated by using the $\Delta\Delta C_T$ (cycle threshold) method and compared with that of the housekeeping gene β-actin.

Immunoblotting

Protein from tissue and culture cells were lysed in RIPA buffer with fresh protease inhibitors. After heat denaturation, 30 μg of total protein was separated on an 8% SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane. After nonspecific blocking, the membrane was probed overnight with rabbit polyclonal anti-Cp110 antibodies (ab99338, 1:1000 dilution; Abcam, Cambridge, Mass) in Tris-buffered saline with 0.2% Tween 20. Blots were visualized by using enhanced chemiluminescence.

Semiquantitative analysis of immunoblots

The immunoblot band intensity signal was quantified with ImageJ software (version 1.44; National Institute of Health, Bethesda, Md). In brief, the area under the curve of the specific signal was corrected for the area under the curve of the loading control (murine β-actin). The value for the control was set at 1, and the other conditions were recalculated correspondingly to allow ratio comparisons.

Scanning electron microscopy

One half of the Transwell insert was removed and processed for immunostaining, as described below. The remaining half of the membranes, left intact in the support, or sinonasal mucosal specimens from patients were fixed with cold 4% polyformaldehyde/2.5% glutaraldehyde overnight and progressively dehydrated in 30%, 50%, 80%, and 100% ethanol at 1-hour intervals. The filters were further processed in 50%, 75%, and 100% hexamethyldisilazane (in ethanol; Electron Microscopy Sciences, Hatfield, Pa) for 1 hour each, followed by overnight incubation in 100% hexamethyldisilazane. The filters were then mounted on scanning electron microscopic (SEM) stubs and sputter coated with gold palladium to a depth of 12 nm, as previously described.[22,23] The surface of the Transwell membrane was examined with an AMR-1400 SEM at an accelerating voltage of 10 kV (Phillips, Amsterdam, The Netherlands). Representative photomicrographs were taken at various angles to effectively display the specimen so that any error in assessment was minimized because of the tilt of the specimen or other artifact.