Autophagy is deficient in nasal polyps: implications for the pathogenesis of the disease

Ling-Feng Wang, MD[1, 2] ,Jeff Yi-Fu Chen, PhD[3], Chen-Yu Chien, MD[1, 4]

[1]Department of Otolaryngology, Faculty of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[2] Department of Otolaryngology, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung, Taiwan
[3]Department of Biochemistry, Kaohsiung Medical University, Kaohsiung, Taiwan
[4]Department of Otorhinolaryngology, Kaohsiung Municipal Hsiao-Kang Hospital, Kaohsiung, Taiwan

Abstract

Purpose: Autophagy has been implicated in many chronic inflammatory diseases including chronic airway inflammation. We will investigate the role of autophagy in the pathogenesis of nasal polyp.
Methods: We studied LC3 protein expression, a common indicators of autophagy, in fresh tissue specimens of five nasal polyps and five control nasal mucosa by Western blot analysis. The results were also confirmed by immunohistochemistry (IHC) using additional twenty-five paraffin-embedded nasal tissue sections. Finally the autophagic activity was validated in nasal polyp-derived fibroblasts by evaluating the number of green fluorescent protein (GFP)-labeled LC3 puncta.
Results: The expression of LC3 was dramatically decreased in all five nasal polyp tissues. In contrast, Akt-mTOR signaling, an established negative regulator of autophagy, was significantly activated in these tissues. Immunohistochemical results further demonstrated a negative correlation between autophagy and nasal polyps ($P < 0.05$). GFP-LC3 puncta formation, an alternative indicator of autophagy, was also diminished in nasal polyp-derived fibroblasts ($P < 0.01$).
Conclusion: Autophagy is deficient presumably due to suppression by high Akt-mTOR activity in nasal polyps, which has offered a novel insight into the mechanism and therapeutics of the disease.

Introduction

Chronic rhinosinusitis with nasal polyposis(CRSwNP) are histologically characterized by increased inflammatory cell infiltration. Various exogenous agents including virus, bacteria, fungi and allergens have been hypothesized to be the etiological factors. However, the initiating event that triggers abnormal and persistent inflammation in CRSwNP remains unidentified[1].

Autophagy is a cellular process that delivers cytoplasmic components to lysosomal degradation for protein and organelle turnover, which is essential for diverse physiological functions such as survival, differentiation, development and homeostasis[2]. However, the role of autophagy in NP pathogenesis is yet to be defined.

The aim of this study was to investigate the expression of autophagy in NPs and control nasal mucosa. We speculated that the autophagic level was drastically reduced in NP tissues and NP-derived fibroblasts possibly due to the activation of Akt-mTOR signaling pathway.

Table Immunohistochemical studies on the expression of LC3 and p-mTOR in nasal tissues

Autophagy markers	Tissues	Staining intensity: Negative + Weak	Moderate + Strong	P value*
LC3	NM	7	10	0.025
	NP	10	2	
p-mTOR	NM	14	3	< 0.001
	NP	2	10	

Abbreviations: NM, control nasal mucosa obtained from bulla ethmoid of CRSsNP patients; NP, nasal polyp of CRSwNP patients.
* Data were analyzed by Fisher's exact test.

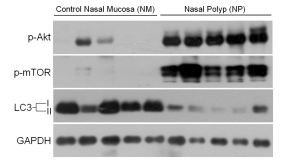

Figure 1. Protein expression of autophagy markers in nasal tissues. Expression of the autophagosomal marker LC3 was significantly decreased in five NP tissues. Conversely, the negative regulators of autophagy signaling p-Akt and p-mTOR were dramatically activated in the same NP tissues. GAPDH was the loading control. The experiments were repeated four times, and the representative data are shown.

Methods and Materials

Patients and Tissues

The study was approved by the IRB of KMUH. The definition of CRSwNP and CRSsNP were based on EPOS [3]. Patients with asthma and other chronic inflammatory diseases were excluded from the study. Nasal polyp tissues (n = 12) and control nasal mucosa (CRSsNP, n = 17) were procured during routine endonasal surgeries.

Primary Culture of Nasal Fibroblasts

The procedures were detailed as our prior study [4]. Primary cultures at the third passages were used in the following experiments.

Tissue Lysates and Western Blot Analysis

Akt-mTOR and LC3 were evaluated. Detailed procedures for Western blot analysis were similar to our prior study [4].

Evaluation of LC3 Puncta Formation

Nasal fibroblasts in 400 ml Opti-MEM® medium (Invitrogen, *Carlsbad*, CA) were transiently transfected with 20 mg GFP-LC3 construct using a BTX ECM 830 electroporator. GFP-LC3 puncta formation was quantitated under a fluorescence microscope.

Immunohistochemistry(IHC)

The intensity of IHC for LC3 and Akt-mTOR was scored based on the percentage of positively stained cells as follows: negative, <5%; weak, 5-35%; moderate, 35-70%; strong, >70%.

Statistical Analysis

LC3 and p-mTOR protein expression and nasal tissues were evaluated using Fisher's exact test. GFP-LC3 puncta formation in nasal tissue-derived fibroblasts was analyzed by Student's t-test. $P < 0.05$ was considered statistically significant.

Results

Inhibition of Autophagy in Nasal Polyp Tissues

As shown in Figure 1, Akt and mTOR, the known negative regulators of autophagy, were highly activated in almost all five NP cases, as the phosphorylation levels of Akt (p-Akt) and mTOR (p-mTOR) were significantly increased in NP tissues compared to those in control mucosa. In sharp contrast, LC3-II expression, the common indicator of autophagic activity, was nearly vanished in the five NP cases, displaying an inverse correlation with the activity of Akt and mTOR.

IHC Staining for Autophagy Markers and the Correlation with Nasal Polyps

In control mucosa, LC3 IHC was primarily present in the cytoplasm of inflammatory leukocytes and stromal cells in submucosa. On the other hand, p-mTOR IHC in NP tissues was localized primarily in the cytoplasm of the epithelium (Figure 2). In contrast to control mucosa, the majority of NP tissues (10/12) had negative to weak LC3 staining intensity (P =0.025). Conversely, most of the NP (10/12) but not NNM (3/17) cases had moderate to strong p-mTOR staining intensity ($P < 0.001$) (Figure 2 and Table).

Reduced Autophagosome Formation in Nasal Polyp-derived Fibroblasts

The number of puncta per cell in NP-derived fibroblasts was significantly lower than that in control mucosa-derived fibroblasts ($P < 0.01$) (Figure 3), suggesting reduced autophagic activity in NP-derived fibroblasts.

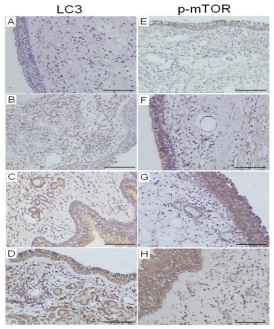

Figure 2. Immunostaining of LC3 and p-mTOR in nasal tissue sections. The representative results are presented for all cases. The left panels show negative staining of LC3 in a NP case (A), and weak (B), moderate (C) and strong (D) staining in control mucosa. The right panels show negative staining of p-mTOR in a control mucosa case (E), and weak (F), moderate (G) and strong (H) staining in NP tissues. Scale bar: 100 μm. Magnification: 200x

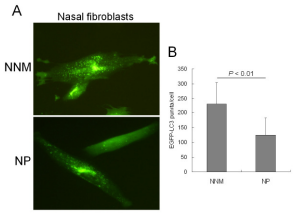

Figure 3. GFP-LC3 puncta formation in nasal tissue-derived fibroblasts. (A) The representative data of GFP-LC3 puncta formation after transfection of GFP-LC3 construct into nasal polyp(NP)- and control nasal mucosa(NM)-derived fibroblasts. (B) The average number of GFP-LC3 puncta per cell was significantly decreased in NP-derived fibroblasts compared to that in control nasal muosa-derived fibroblasts. GFP-LC3 puncta number in fourteen different cells was manually counted under a fluorescence microscope, and the results were expressed as mean ± SD.

Discussion

In this study, we showed that autophagy was largely suppressed in NPs presumably due to the highly activated Akt-mTOR signaling pathway. Currently how mechanistically autophagy deficiency may affect NP pathogenesis is unknown. Several human inflammatory diseases have been associated with autophagy. [5-8] More interestingly, there are previous studies indicating an inverse correlation between autophagy and inflammation in different cell contexts, [6,9,10] raising the possibility that autophagy might impact nasal polyposis through modulating inflammatory responses.

Conclusions

In summary, to our knowledge, this study has demonstrated the first implication that autophagy could be involved in the molecular mechanism of NP pathogenesis. Further study is needed to investigate its possible mechanism.

Contact

Ling-Feng Wang, MD
Department of Otolaryngology Head and Neck Surgery, Kaohsiung Medical University
Department of Otolaryngology, Kaohsiung Municipal Ta-Tung Hospital, Kaohsiung Medical University
Email: lifewang@kmu.edu.tw
886-7-3121101-5009

References

1. Settipane RA, Peters AT, Chiu AG. Chapter 6: Nasal polyps. Am J Rhinol Allergy. 2013;27 Suppl 1:S20-25.
2. Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell. 2008; 132:27-42.
3. Fokkens W, Lund V, Mullol J. EP3OS 2007: European position paper on rhinosinusitis and nasal polyps 2007. A summary for otorhinolaryngologists. Rhinology. 2007; 45:97-101.
4. Chen JY, Hung CC, Huang KL, et al. Src family kinases mediate betel quid-induced oral cancer cell motility and could be a biomarker for early invasion in oral squamous cell carcinoma. Neoplasia. 2008; 10:1393-1401.
5. Alirezaei M, Kemball CC, Whitton JL. Autophagy, inflammation and neurodegenerative disease. Eur J Neuroscience. 2011; 33:197-204.
6. De Luca A, Smeekens SP, Casagrande A, et al. IL-1 receptor blockade restores autophagy and reduces inflammation in chronic granulomatous disease in mice and in humans. Proc Natl Acad Sci U S A. 2014; 111:3526-3531.
7. Hall LJ, Watson AJ. Role of autophagy in NOD2-induced inflammation in Crohn's disease. Gastroenterology. 2012; 142:1032-1034.
8. Nakahira K, Cloonan SM, Mizumura K, Choi AM, Ryter SW. Autophagy: a crucial moderator of redox balance, inflammation, and apoptosis in lung disease. Antiox Redox Signaling. 2014; 20:474-494.
9. Chen ML, Yi L, Jin X, et al. Resveratrol attenuates vascular endothelial inflammation by inducing autophagy through the cAMP signaling pathway. Autophagy. 2013; 9:2033-2045.
10. Yoshizaki T, Kusunoki C, Kondo M, et al. Autophagy regulates inflammation in adipocytes. Biochem Biophys Res Commun. 2012; 417:352-357.