HDM-induced Atg5 Mediates TSLP Expressing to Disrupt Epithelial Barrier

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Research

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Abstract

Background: Asthma is a complex and heterogeneous disease. Autophagy, process of self-protection in cells, is an intracellular process when cells are being attacked by certain stress. Our team focused upon the disruption of airways epithelial barrier in asthma, and we interested in whether autophagy played a key role in asthma.

Methods: 400U HDM was used to treat HBECs and established asthmatic mice model. Western blotting, RT-PCR and immunofluorescence were mainly used to detect autophagy process in vivo and in vitro. One way ANOVA and Mann Whitney test were used for statistic.

Results: After treated with HDM, expression of LC3ab increased in vivo and in vitro. Using Rapamycin, 3-MA and Chloroquine to treat HBECs, then we surprisingly found that HDM disrupts epithelial barrier through incomplete autophagy. To find out the connection between asthma and autophagy, we chose known autophagy related genes to determine the association between autophagy and disruption of airway epithelial barrier. Atg5 and atg12 were chosen because these two genes varied upon the time dependent manner. Knocked down the expression of atg5 or atg12 by siRNA, the expression of TSLP, which can induce the disruption of airway epithelial barrier, remarkably reduced.

Conclusions: These results demonstrated that HDM induced inflammatory in airway epithelium through autophagy, and then knocked down autophagy related genes alleviated the inflammatory in HBECs.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

Fig 1

Figure 1

Autophagy could be induced by treatment of HDM both in vivo. A. H&E staining mice lung sections with HDM stimulation (400 × magnification). B. PAS staining mice lung sections with HDM stimulation (400 × magnification). C. Airway hyper-responsiveness was measured by PFT Pulmonary Maneuvers. D. Immunohistochemistry (IHC) staining image of LC3ab and P62 in mice lung sections. Data are presented as the mean±SD; n≥3. *P<0.05, **P<0.01, ***P<0.0001.

Fig 2

Figure 2

HDM induced autophagy in vitro. A. Western blotting analysis of LC3ab and P62 in HBECs which were stimulated with HDM (400U) for 24h. B. CCK8 analysis of HBECs in 24h stimulated with Rapamycin, 3-MA and CQ respectively. C. Western blotting analysis of P62 and LC3ab in HBECs stimulated with or without HDM for 24h and treated with Rapamycin, 3-MA and CQ respectively. D. HBECs that expressed mRFP-EGFP-LC3 fusion protein were stimulated with HDM (400U). Confocal microscopic analysis is shown

(1000 × magnification). Data are presented as the mean±SD; n≥3. *P≤0.05, **P≤0.01, ***P≤0.0001 vs to control, #P≤0.05, ##P≤0.01, ###P≤0.0001 vs to HDM.

Fig 3

Figure 3

HDM disrupted airway epithelial barrier through autophagy. A. The FITC-Dx permeability was measured to determine epithelial barrier integrity after the HBECs treated with or without HDM (400U) and with Rapamycin, 3-MA and CQ relatively for the indicated times. B. Transepithelial electrical resistance (TEER)

was measured to determine epithelial barrier integrity after the HBECs treated with or without HDM (400U) and with Rapamycin, 3-MA and CQ relatively for the indicated times. C. The distribution of E-cadherin and β -catenin was monitored using immunofluorescence. Green represents E-cadherin, red represents β -catenin, blue represents the nucleus. Confocal microscopic analysis is shown (1000 $\times$ magnification). D. Western blotting analysis of E-cadherin and β -catenin in HBECs stimulated with or without HDM for 24h and treated with Rapamycin, 3-MA and CQ respectively. E. Western blotting detected the expressions of E-cadherin and β -catenin in membrane in HBECs stimulated with or without HDM for 24h and treated with Rapamycin, 3-MA and CQ respectively (ATP1A1 for membrane, β -tubulin for cytoplasm, Lamin B for nucleus). Data are presented as the mean $\pm$ SD; n $\geq$ 3. *P $\leq$ 0.05, **P $\leq$ 0.01, ***P $\leq$ 0.0001 vs to control, #P $\leq$ 0.05, ##P $\leq$ 0.01, ###P $\leq$ 0.0001 vs to HDM.

A

HDM induced autophagy through Atg5 & Atg12 in HBECs. A. Western blotting analysis of LC3ab in HBECs stimulated with HDM(400U) for indicated times. B. Western blotting analysis of Atg7, Atg16L1, Atg12 and Atg5 in HBECs stimulated with HDM (400U) for indicated times. C. qPCR assay of Atg7, Atg16L1, Atg10, Atg12 and Atg5 mRNA in HBECs stimulated with HDM (400U) for indicated times. Data are presented as the mean±SD; n≥3. *P<0.05, **P<0.01, ***P<0.0001.

Fig 5

Figure 5

The mRNA level of TSLP was mediated by Atg5 or Atg12. A. Immunohistochemistry (IHC) staining image of TSLP, IL-25 and IL-33 in mice lung sections. B. qPCR assay of Atg5 and Atg12 mRNA in HBECS treated with siATG5 & siATG12 respectively. C. qPCR assay of TSLP, IL-33 and IL-25 in HBECS with or without siATG5 and siATG12. Data are presented as the mean±SD; n≥3. *P<0.05, **P<0.01, ***P<0.0001 vs to control, #P<0.05, ##P<0.01, ###P<0.0001 vs to HDM.

Fig 6

A

B

C

Figure 6

Expression of TSLP was blocked after interfering Atg5 but not Atg12. A. Western blotting analysis of Atg5 or Atg12 in HBECS treated with siATG5 and siATG12 respectively. B. Western blotting analysis of HBECS treated with siATG5 and with or without HDM (400U). C. Western blotting analysis of HBECS treated with siATG12 and with or without HDM (400U). Data are presented as the mean±SD; n≥3. *P<0.05, **P<0.01, ***P<0.0001 vs to control, #P<0.05, ##P<0.01, ###P<0.0001 vs to HDM.