学 位 論 文 の 要 旨

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学 位 論 文 名　Notch Signaling Pathway and Cdx2 Expression in the Development of Barrett's Esophagus

発 表 雑 誌 名（巻，初頁～終頁，年）　Laboratory Investigation 92: 896-909, 2012

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論 文 内 容 の 要 旨

INTRODUCTION

Cdx2 expression in esophageal stem cells induced by reflux bile acids may be an important factor for development of Barrett's esophagus, while Notch signaling is a fundamental molecular signaling system that governs cell fate decisions such as differentiation and proliferation in numerous tissue types. Notch ligands binding to Notch receptors induce the cleavage of Notch receptor by γ-secretase to release Notch intracellular domain (NICD), which translocates from the cell membrane to the nucleus, thereby functioning as a transcriptional activator of target genes such as a *Hes1*, and downstream target gene *ATOH1* is critically suppressed. Cdx2 is well-known factor for inducing intestinal metaplasia. ATOH1 is also important factor to induce goblet cell differentiation. It has been reported that Cdx2 induces ATOH1 expression by blocking Notch signaling, and then ATOH1 plays an important role in the differentiation of stem cells into goblet cells. However, the relationship between Notch signaling and Cdx2 expression in the development of goblet cell metaplasia in Barrett's esophagus remains unclear. To examine this potential relationship, we developed the following hypotheses. ATOH1 is induced in the Barrett's esophagus with goblet cell metaplasia. And Cdx2 induced by reflux bile acids is closely related with ATOH1 expression. The aim of this study was to investigate the interrelationship between Notch signaling and Cdx2 in esophageal epithelial cells.

MATERIALS AND METHODS

First, we evaluated the basal expression of Notch signaling molecules, such as Noth1, Hes1, and ATOH1, in 51 esophageal columnar epithelia with or without goblet cell metaplasia. These expressions were examined by real-time polymerase chain reaction (PCR) and immunohistochemical staining using the biopsy specimen taken from esophageal intestinal metaplasia (IM) with goblet cells [IM(+)] and columnar epithelium not accompanied by goblet cells [IM(-)]. The study protocol was prepared according to the Declaration of Helsinki and approved by the ethics committee of Shimane University, Faculty of Medicine. Written informed consent was obtained from all patients.

Next, for *in vitro* experiments, we employed human esophageal epithelial cell lines (OE33, OE19, and Het-1A). After forced Cdx2 expression by applying a Cdx2 expression vector to the cells, changes in the expressions of Notch1, Hes1, ATOH1, Cdx2, and MUC2 were analyzed by real-time PCR and Western blot analyses. The changes in expressions of Notch1, Hes1, ATOH1, Cdx2, and MUC2 in the cells were analyzed by real-time PCR and Western blot analyses following stimulation with bile acids [deoxycholic acid (DCA) or cholic acid (CA)] in the presence or absence of Cdx2 blocking with *Cdx2*-siRNA.

RESULTS AND DISCUSSION

Suppressed *Hes1*, and enhanced *ATOH1* and *MUC2* mRNA expressions were identified in IM(+) specimens. The results of immunohistochemical staining also revealed significantly enhanced Cdx2 and ATOH1 expressions in IM (+), suggesting that Notch signaling was inhibited in IM (+).

Hes1 mRNA was suppressed and *ATOH1* and *MUC2* mRNA were enhanced significantly in Cdx2 expression vector treated cells. Consistently, the results of Western blot analysis provided similar results. These results suggest that enhanced expression of Cdx2 showed decreased Notch signaling pathway. Likewise, bile acids augmented *ATOH1* and *Cdx2* mRNA expressions in time and dose dependent manners in all cell lines, while *MUC2* mRNA expression was consistently augmented by bile acids in the cells. In addition, *Hes1* mRNA expression was suppressed by stimulation with bile acids in time and dose dependent manners in the cells. Consistently, the results of Western blot analysis provided similar results. These results point out the possibility that blockade of Notch signaling due to the enhanced expression of Cdx2 by stimulation with bile acids for the development of goblet cell metaplasia in Barrett's esophagus.

So, next we focused the relationship Notch signaling and Cdx2 expression. On the other hand, in *Cdx2*-siRNA treated cells, bile acids did not change Hes1 and ATOH1 expression. These results suggest that blocking of Notch signaling by bile acids is Cdx2 dependent.

CONCLUSION

These results indicate that over-expression of the transcription factors Cdx2 and ATOH1 induced by bile acids may change the phenotype of esophageal stem cells into columnar cells. We found that induction of ATOH1 expression via Hes1 suppression in esophageal epithelial cells in response to bile acids has important functions in induction of metaplastic changes during Barrett's epithelium development. In addition, our results revealed that the transcriptional network related to the Notch signaling pathway and Cdx2 also has important roles in that development.