Review Article
The Role of Atoh1 in Mucous Cell Metaplasia

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A key issue in otitis media is mucous cell metaplasia which is responsible for mucous hypersecretion and persistence of the disease. However, little is known about the molecular mechanisms of mucous cell metaplasia in otitis media. Numerous studies of intestinal epithelial homeostasis have shown that Atonal homolog 1 (Atoh1), a basic helix-loop-helix (bHLH) transcription factor, is essential for the intestinal goblet cell differentiation. On the other hand, SAM-pointed domain-containing Ets transcription factor (SPDEF), a member of the "Ets" transcription factor family, has been reported to trigger the mucous cell metaplasia of pulmonary infectious diseases or asthma. Recent studies have demonstrated the relation of these factors, that is, Spdef functions downstream of Atoh1.

We could take the advantages of these findings for the study of otitis media because both middle ear and pulmonary epithelia belong to the same respiratory tract. Atoh1 and SPDEF could be the therapeutic targets for otitis media associated with mucous cell metaplasia which is frequently considered “intractable” in the clinical settings.

1. Mucous Cell Metaplasia in Intractable Otitis Media

One of the most characteristic features of intractable otitis media is mucous hypersecretion. Chronic otitis media, caused by a prolonged bacterial infection, is characterized by ear discharge that is rich in mucus. Otitis media with mucoid effusion, common in young child otitis media patients and caused by a combination of bacterial infection and dysfunction of the Eustachian tube [1], is characterized by thick fluid accumulation in the middle ear cavity. Eosinophilic otitis media, which is a newly recognized intractable otitis media with bronchial asthma, is characterised by the presence of a highly viscous yellow effusion containing eosinophils [2].

The pathological basis of the mucous hypersecretion is mucous cell metaplasia, abnormal transformation of the normal respiratory tract mucosa into a thickened mucosa with a high density of the goblet cell population in response to microorganic infections or asthma. In a murine model of asthma, there was a dramatic increase in goblet cell number accompanied by a 75% decrease in Clara cells and a 25% decrease in ciliated cells after allergen challenge with a total number of epithelial cells being constant [3]. The increase of goblet cells and decrease of ciliated cells are also reported in the rat model of otitis media with effusion induced by the endotoxin plus Eustachian tube obstruction [4]. These findings suggest that the ciliated cells convert to goblet cells under the pathological conditions via a biologic process called cell transdifferentiation. It has been demonstrated that various materials such as bacteria [1], lipopolysaccharide [5], tumor necrosis factor-alpha (TNF-α) [6], and interleukin-10 (IL-10) [7] are involved in mucous cell metaplasia of the middle ear mucosa.

Mucins serve critical functions in the host defense against microorganisms. They are the principal structural components of the mucociliary transport system and the
pathogens entrapped in a mucin gel layer are eliminated by constant beatings of the cilia on the mucosal surface. Under normal conditions, few mucous cells exist in the middle ear mucosa and they are mainly distributed in the orifice of the Eustachian tube, promontory area, and inferior tympanium. However, under pathological conditions inducing mucous cell metaplasia, an increased goblet cell population in the mucosa produces excessive mucus, while the loss of ciliated cells in the mucosa results in the dysfunction of the mucociliary transport system, leading to subsequent problems such as fluid accumulation and repeated infections. It is necessary to understand the disease mechanism of mucous cell metaplasia in order to cure this intractable illness.

2. The Role of Atoh1 in Mucous Cell Differentiation

Atonal homologue 1 (Atoh1), also called the mouse atonal homolog 1 (Math1) gene in mice and the human atonal homolog 1 (Hath1) gene in humans, is one of the candidate genes which play a crucial role in the mucous cell metaplasia. Atoh1 is a basic helix-loop-helix (bHLH) transcription factor that is essential for the intestinal goblet cell differentiation. It has been shown that goblet cells are completely abrogated in the intestine of Math1-null mouse [8]. Math1-null embryos die at birth due to respiratory failure and lack of many specific cell lineages, including not only intestinal secretory cells but also cerebellar granule neurons, spinal cord interneurons, and inner ear hair cells [9].

The role of Atoh1 in goblet cell differentiation has been well studied in the intestine. The epithelium of the small intestine is composed of four different cell types: absorptive enterocytes and three secretory lineages consisting of mucous-secreting goblet cells, hormone-secreting enteroendocrine cells, and antimicrobial peptide-secreting Paneth cells. The intestinal epithelium is a highly dynamic tissue and is replaced by newly differentiated cells from multipotent stem cells residing near the base of the Crypts in every 2–7 days [9]. The differentiation for an absorptive or secretory cell lineage is controlled by the Notch signaling system in a manner of lateral inhibition (Figure 1). Notch is a cell surface receptor that binds to Notch ligands, Dll (Delta-like), and Jagged families. Notch receptor activation prevents Atoh1 expression and triggers goblet cell differentiation.

3. The Role of SPDEF in Mucous Cell Metaplasia

SPDEF (SAM Pointed Domain ETS Factor) is another candidate controlling mucous cell metaplasia. It is a member of the “Ets” family which regulates a number of biological processes, including cell proliferation, differentiation, and invasion. SPDEF was first described as a factor interacting with the androgen receptor to enhance expression of the prostate-specific antigen (PSA) promoter in vitro [15].

The role of SPDEF in mucous cell metaplasia is well attended in the study of lung diseases. SPDEF was markedly increased at sites of mucous cell metaplasia in bronchial tissues from patients with Cystic fibrosis or cigarette smoking [16]. In a murine model of asthma, the expression of...
SPDEF was also increased at sites of mucous cell metaplasia caused by IL-13 and dust mite allergen [17]. Chen et al. [16] have shown the Clara cell turns to goblet cell in the lung within 3 days after expression of SPDEF using a transgenic mouse model where the expression of Spdef gene under the Clara cell-specific promoter is controlled by doxycycline concentration (Scgb1a1-rtTA/TRE2-Spdef). This process is rapidly reversible and associated with the restoration of the Clara cells after stopping of the SPDEF expression. They have also reported some genes under the control of SPDEF. An mRNA microarray analysis study showed the expression of SPDEF influenced more than 300 genes including mucin 16 (MUC16), anterior gradient 2 (AGR2), glucosaminyl (N-acetyl) transferase 3, mucin type (Gcnt3), and chloride channel calcium activated 1 (Clca1). The involvement of these genes was confirmed by others. MUC5AC, AGR2, and Clca1 induced by IL-13 treatment were reduced in Spdef knockout human airway epithelial cells [18]. In colon cancer cells to activate the goblet cell genes by Notch signal inhibitors, knockdown of Spdef also repressed the expression of MUC2 and AGR2 [19]. Mucin is a large-molecular-weight glycoprotein and mucin production requires several steps including transcription of a MUC gene, holding, multimerization, and glycosylation. MUC genes code core mucin protein. AGR2 is a member of protein disulfide isomerase (PDI) family which is critical for efficient formation of correctly arranged disulfide bonds in the endoplasmic reticulum (ER) [20]. Gcnt3 is involved in the synthesis of a core structure in the mucin glycan chain. Clca1, whose mouse homolog is called as Gob5, is believed to have a role in secreting chloride anions into the lumen and contributing to the salt and water composition of secreted mucins [21]. All these genes contribute the production of mature mucins, which is conducted in the well-differentiated goblet cells. Inversely, blockage of SPDEF results in a failure of goblet cell maturation. The intestine of Spdef knockout mice revealed a severe loss of mature goblet cells and Paneth cells accompanied by accumulation of immature secretory progenitors. The typical aberrant goblet cells in Spdef knockout mice exhibit a clear brush border similar to adjacent enterocytes and carry poorly defined vacuoles in their cytoplasm. These immature goblet cells probably initiate the differentiation of goblet cells because they expressed trefoil factor 3 (TFF3), which is one of the goblet cell markers and thought to help in the oligomerization of mucin polysaccharides, although they did not have Alcian Blue Periodic Acid–Schiff (AB-PAS)-positive granules [22]. These data suggest SPDEF is a factor to serve for the terminal differentiation of goblet cells rather than to initiate or trigger the differentiation of goblet cells. SPDEF is thought to be a key transcription factor to start a series of sequential gene activations for the terminal differentiation and maturation of goblet cells.

4. SPDEF Functions as a Downstream Molecule of Atoh1

Accumulated pieces of evidence indicate that Spdef functions downstream of Atoh1, and this is mediated by another transcription factor, growth-factor-independent 1 (GFI1), which is normally expressed in the both Paneth and goblet cells (Figure 2). SPDEF was absent from intestine of the Math1-null mouse and was also quantitatively reduced in the GFI1-null mouse [19]. GFI1 is thought to be downstream of Atoh1 because the Math1 expression was still observed in the GFI1-null mouse [9]. Atoh1-null crypts lack all the intestinal
secretory cells. On the other hand, GFI1-null crypts lack Paneth cells, have few goblet and supernumerary enteroendocrine cells. These findings indicate the selection between secretory cells (goblet cells, Paneth cells, and enteroendocrine cells) and absorptive cell is dependent on Atoh1, and the selection between goblet/Paneth and enteroendocrine cells is dependent on GFI1 [9].

5. The Role of Atoh1 in Otitis Media

There is no report to discuss the involvement of Atoh1, GFI1, or Spdef in otitis media. However, the similarity of the middle ear epithelium to the pulmonary epithelium, Atoh1, the upstream gene of Spdef which is involved in several pulmonary diseases, may also have a role in the mucous cell metaplasia of otitis media. To test this hypothesis, we performed the transfection of Math1 in the mouse middle ear. It was found that mucous cell numbers were significantly increased in Math1 transfected middle ears compared with empty vector transfection (Figure 3). We also evaluated the effects of administration of TNFα, which is a common proinflammatory cytokine induced by bacterial or virus infection, and retinoic acid (RA) on mouse middle ear epithelial cells [23]. The administration of these factors for two weeks synergistically promoted the expression of MUC2 and TFF3 with an activation of Math1 in vitro (Figure 4). These data suggest that Atoh1 activated by proinflammatory cytokines triggers the mucous cell metaplasia in the middle ear epithelium as well.

6. Conclusions

Over the past decade there have been significant advances in the studies of intestinal epithelial homeostasis and pulmonary mucous cell metaplasia. The former studies found Atoh1 as a factor to trigger the differentiation of goblet cells, and the latter studies found SPDEF as a factor to be responsible for mucous cell metaplasia, respectively. Now we can take advantages of these molecular understanding about mucous cells for the therapy of otitis media. Mucous cell metaplasia is a very primitive response to pathogens. Originally, this response is to protect the mucociliary system by secreting more mucins and their chaperones. These secreted substances are supposed to lubricate the lumen of the respiratory tract and discharge hazardous materials. However, mucous cell metaplasia also disturbs the well-organized mucociliary transport system at the expense of ciliated cells. Excessive mucus severely impairs the function of the mucociliary transport system and leads to subsequent infections. To make matter worse, bacterial cell wall components, or inflammatory cytokines trapped in accumulated mucus can be a driving force again for mucous cell metaplasia and thus form a vicious cycle. This vicious circle could explain why mucous cell metaplasia prolongs infectious diseases on a long-term basis. The effective blockage of the Atoh1, SPDEF, or GFI1 expression in the early phase of disease may be a new strategy for treating intractable otitis media. It could break the vicious circle of mucous cell metaplasia and lessen the burden of otitis media patients with mucoid effusion.

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