Human neutrophil elastase-mediated goblet cell metaplasia is attenuated in TACE-deficient mice

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Park JA, Sharif AS, Shiomii T, Kobzik L, Kasahara DI, Tschumperlin DJ, Voynow JA, Drazen JM. Human neutrophil elastase-mediated goblet cell metaplasia is attenuated in TACE-deficient mice. Am J Physiol Lung Cell Mol Physiol 304: L701–L707, 2013. First published April 5, 2013; doi:10.1152/ajplung.00259.2012.—Neutrophilic inflammation is associated with chronic airway diseases. It has been observed that human neutrophil elastase (HNE), which is secreted by active neutrophils during inflammation, induces both mucin overproduction and goblet cell metaplasia. Several in vitro studies suggest that tumor necrosis factor-α converting enzyme (TACE) regulates the signaling axis that mediates HNE-induced mucin overproduction; however, it is unknown whether TACE performs a similar function in HNE-induced goblet cell metaplasia in vivo. We conducted this study to determine whether the inactivation of Tace gene expression attenuates HNE-induced goblet cell metaplasia in mice. Deletion of Tace is lethal shortly after birth in mice; therefore, we utilized Taceflox/floxR26CreER+/− mice and induced conditional deletion of Tace using a tamoxifen injection. Wild-type mice were given tamoxifen to control for its effect. We found that HNE induced goblet cell metaplasia in the wild-type mice and that HNE-induced goblet cell metaplasia was significantly attenuated in the Tace conditional deletion mice. These findings suggest that TACE could be a potential target in the treatment of goblet cell metaplasia in patients with chronic airway diseases.

Neutrophilic inflammation

Neutrophilic inflammation is associated with chronic airway diseases such as chronic obstructive pulmonary disease, cystic fibrosis, and asthma (6, 24, 33). Mediators are secreted by active neutrophils during inflammation; one such mediator is neutrophil elastase (20). It has been observed that human neutrophil elastase (HNE) induces the expression of mucin genes such as MUC1, MUC4, and MUC5AC (8, 9, 15, 29) and the secretion of MUC5AC and MUC5B (13, 22) in various epithelial cells in vitro. Under normal conditions, mucin plays a protective role in the airway. When HNE induces the expression of mucin genes in patients with chronic airway diseases (7), mucin overproduction causes airway obstruction and enhanced bacterial colonization that promote chronic inflammation and impaired mucociliary clearance due to increased viscosity of mucus (26, 31).

Voynow and her colleagues (1, 10, 32) established a mouse model showing that HNE induces goblet cell metaplasia (GCM) in the airway, which results in mucin hypersecretion and overproduction. They found that the repeated exposure to HNE in mice induced GCM and mucin gene expression. They also determined that the proteolytic activity of HNE mediates HNE-induced GCM by observing that the administration of the HNE inhibitor Ala-Ala-Pro-Val chloromethylketone attenuates HNE-induced GCM. Additionally, Arai et al. (1) found that HNE-induced GCM is attenuated by a long-acting cholinergic antagonist, tiotropium.

Although these studies clearly show that HNE induces GCM, the underlying cellular signaling mechanism that enables HNE to induce GCM has not yet been fully elucidated in vivo. However, extensive studies performed in vitro have revealed that HNE-induced mucin gene expression is associated with the activation of the protein kinase C (PKC)-tumor necrosis factor-α (TNF-α) converting enzyme (TACE) pathway. TACE, also known as a disintegrin and metalloproteinase 17 (ADAM17), is a membrane-anchored enzyme that cleaves cell-surface proteins including epidermal growth factor receptor (EGFR) ligands, cytokines and their receptors, and adhesion molecules (2, 27). Among EGFR ligands, amphiregulin has been shown to contribute to GCM that occurs during naphthalene-induced lung injury in mice (18).

Furthermore, activation of the PKC-TACE-EGFR signaling cascade mediates HNE-induced MUC5AC expression and MUC5AC secretion in NCI-H292 cells (29) and activation of the PKC-TACE-tumor necrosis factor receptor 1 (TNFR1) signaling cascade mediates HNE-induced MUC1 expression in A549 cells (15).

Because TACE regulates the signaling axis that mediates HNE-induced mucin overproduction in vitro, we hypothesized that TACE plays an important role in HNE-induced GCM in vivo. In this study, we used Taceflox/floxR26CreER+/− mice to demonstrate that deletion of TACE attenuates HNE-induced GCM in mice, which suggests that TACE plays a pivotal role in the regulation of GCM and could be a potential therapeutic target in chronic airway diseases.

Materials and Methods

Animals. R26CreER+/− [B6;129-Gt(Rosa)26Sortm1(Cre/ERT2)Nadl] mice were purchased from Jackson laboratory (Bar Harbor, ME). Taceflox/floxR26CreER+/− and Taceflox/floxR26CreER+/− mice were previously generated by crossing Taceflox/flox with R26CreER+/− (30). In Taceflox/flox mice loxP sites flank exon 2 (11). Mice at ~8–12 wk of age were used for all experiments.

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Conditional deletion of Tace was generated by tamoxifen injection. Tamoxifen was prepared as a 10 mg/ml stock solution in sunflower oil containing 5% ethanol and was injected intraperitoneally (IP) daily for 5 days. On day 7 after the last tamoxifen injection, mice were instilled intranasally with HNE (50 µg in 40 µl of PBS) or 40 µl of PBS 3 times at 3-day intervals. On day 4 after the last HNE exposure, lungs were harvested for histology and mRNA analysis of MucSac.

Fig. 1. Experimental procedure for tamoxifen injection and human neutrophil elastase (HNE) exposure. Tamoxifen was injected intraperitoneally (IP) daily for 5 days. On day 7 after the last tamoxifen injection, mice were instilled intranasally with HNE (50 µg in 40 µl of PBS) or 40 µl of PBS 3 times at 3-day intervals. On day 4 after the last HNE exposure, lungs were harvested for histology and mRNA analysis of MucSac.

Table 1. Primers for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer (5′→3′)</th>
<th>Reverse Primer (5′→3′)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Gapdh</td>
<td>CARGGCGCTTCGGGATTCTTA</td>
<td>TGCTTCAAGGACCTTCTGATG</td>
<td>30</td>
</tr>
<tr>
<td>MucSac</td>
<td>AGAGAGGGGTTGAGCTGGTTT</td>
<td>CTCTGCGGCTCTGCTGATGCT</td>
<td>32</td>
</tr>
</tbody>
</table>
R26CreER<sup>+/−</sup> mice (Tace conditional deletion mice) and Tace<sup>lox/lox</sup>R26CreER<sup>−/−</sup> mice (wild-type mice). Both genotypes received five consecutive tamoxifen injections to ensure that depletion of TACE was not caused by the toxicity of tamoxifen. In pilot experiments (not shown), depletion of TACE was detectable from day 3 after the last tamoxifen injection.

Tace mRNA expression was also determined by real-time PCR. The primers used for real-time PCR spanned Tace exon 2, which is removed upon activation of Cre because of the loxP sites flanking that exon. Tace mRNA expression in the Tace conditional deletion mice was 12.67% of that in the wild-type mice (Fig. 2A). Although the pro and mature forms of TACE protein were both detected in the lung lysates of both genotypes, the level of mature TACE was much higher in the wild-type mice than in the Tace conditional deletion mice after tamoxifen injection (Fig. 2B).

Goblet cell metaplasia in mice exposed to HNE. We collected the lungs on study day 11 and used PAS staining to determine the goblet cell index in four sample groups: wild-type mice and Tace conditional deletion mice exposed to PBS (control groups), and wild-type mice and Tace conditional deletion mice exposed to HNE. The goblet cell index ranges from 0 to 4, with 4 indicating the highest concentration of goblet cells, as determined through blinded scoring performed by an investigator with anatomic pathology training and experience.

Under normal conditions, mice express a limited number of goblet cells in the lungs. The lungs of the wild-type mice and the Tace conditional deletion mice that were exposed to PBS had a goblet cell index level of 0, and there was no detectable difference in the number of goblet cells at the basal level between the two genotypes (Fig. 3, A and C).

GCM was induced in the wild-type mice that were exposed to HNE (Fig. 3B), compared with the mice that were exposed to PBS; the goblet cell index level of these airways ranged from 0 to 4, with a median of 1.5 (Fig. 3E). However, in the Tace conditional deletion mice, HNE-induced GCM was significantly attenuated (Fig. 3D); the goblet cell index level ranged from 0 to 2, with a median of 0.4 (Fig. 3E) (<i>P</i> < 0.05, compared with wild-type mice).

To confirm that the PAS-positive cells were truly MUC5AC-positive cells, we performed immunohistochemical staining on the lung tissue samples using a monoclonal antibody against MUC5AC. Tissue from wild-type mice exposed to HNE, which was PAS positive, consistently showed MUC5AC-positive staining as well (representative image shown in Fig. 4A). In contrast, we did not detect MUC5AC-positive cells in the Tace conditional deletion mice that were exposed to HNE (representative image shown in Fig. 4B).

We also measured the expression of Muc5ac mRNA on day 4 after the last HNE exposure (day 11 after the initial HNE exposure). There was a slight induction of Muc5ac mRNA expression in the wild-type and Tace conditional deletion mice after HNE exposure, although this failed to reach statistical significance in either genotype. In addition, there was no significant difference in the transcript levels of Muc5ac between the two genotypes, in contrast to the clearly different levels of goblet cells present on day 11 after the initial HNE exposure (Fig. 4C).

Tissue inflammation in mice exposed to HNE. As a secondary outcome, we performed H&E staining on the lung tissues to determine the level of inflammation in the mice exposed to HNE. The degree of inflammation was scored again in a blinded fashion; the scoring ranged from 0 to 5, with 5 indicating the highest level of inflammation. The range of inflammation was 0 to 2 in both the wild-type mice and the Tace conditional deletion mice (Fig. 5). There was no significant difference between the genotypes.

We also quantified the concentration of TNF-α in BAL collected from mice on the same day as we performed other observations. Surprisingly, TACE deletion did not attenuate the TNF-α levels in BAL fluid, as shown in Fig. 5C.

**DISCUSSION**

We conducted this study to determine whether the conditional deletion of Tace attenuates HNE-induced GCM in vivo. The strength of our study is the Cre-loxP conditional deletion system we utilized, which allowed us to study a
gene deletion that otherwise results in perinatal lethality (23). We confirmed that HNE exposure in wild-type mice led to GCM, as measured by PAS and MUC5AC staining, and we now report that HNE-induced GCM is significantly attenuated in \( \text{Tace}^{\text{flox/flox}}/\text{Cre}^{-/-} \) mice (\( \text{Tace} \) conditional deletion mice), a finding that affirms our hypothesis that TACE is a regulator of HNE-induced GCM in vivo.

Despite definitive histological evidence of GCM and positive MUC5AC immunostaining, we did not observe significant changes in \( \text{Muc5ac} \) mRNA expression at day 11, 4 days after the last HNE exposure. This is likely the result of transient changes of message level returning to baseline at 4 days after the last HNE exposure. Alternatively, the difference between MUC5AC immunostaining and \( \text{Muc5ac} \) mRNA expression could be accounted for by a decrease in mucin secretion or an increase in mucin synthesis in \( \text{Tace} \) conditional deletion mice. In addition to GCM, infiltration of inflammatory cells is often present in patients with chronic airway diseases and has been previously noted in response to HNE exposure (32). However, at day 4 after the last HNE exposure (day 11 after the first HNE exposure) an assessment of tissue inflammation, using H&E staining to visualize infiltrating inflammatory cells, showed indistinguishable levels of tissue inflammation in \( \text{Tace} \) conditional deletion mice and control mice. Similarly, TNF-\( \alpha \) levels were indistinguishable in the BAL fluid obtained from wild-type and \( \text{Tace} \) conditional deletion mice. These results suggest the conditional deletion of \( \text{Tace} \) acted selectively on GCM without altering the inflammatory response to HNE, although caution is warranted in interpreting our findings because a previous study by Voynow et al. (32) using the same HNE exposure noted a maximal inflammatory response at day 8 that was greatly diminished by day 11 after the first HNE exposure.

TNF-\( \alpha \) is an important proinflammatory cytokine that is synthesized as a membrane-anchored precursor form and shed by TACE. TNF-\( \alpha \) has previously been linked to mucin gene expression in vitro and in vivo (4, 15), making it a candidate to mediate the functional effects of the TACE deletion observed in our study. However, our analysis of BAL fluid revealed that TNF-\( \alpha \) levels were increased after HNE exposure in both wild-type and \( \text{Tace} \)-deficient mice, in contrast to the divergent effects of HNE on goblet cell

![Fig. 3. Periodic acid-Schiff (PAS) staining of the airway. A–D: PAS staining was performed to visualize goblet cells in the lungs harvested on day 11 after the first exposure to HNE. \( \text{Tace}^{\text{flox/flox}}/\text{Cre}^{-/-} \) mice (wild-type mice) that were instilled with HNE developed goblet cell metaplasia, but HNE-mediated goblet cell metaplasia was attenuated in \( \text{Tace}^{\text{flox/flox}}/\text{Cre}^{-/-} \) mice (\( \text{Tace} \) conditional deletion mice). PBS instillation was used as a negative control and did not induce goblet cell metaplasia in either genotype. E: the goblet cell index determined by PAS staining shows that HNE significantly increased the number of goblet cells in the airways of wild-type mice (\( n = 10 \)) compared with \( \text{Tace} \) conditional deletion mice (\( n = 14 \)).](http://ajplung.physiology.org/)

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metaplasia observed in these two genotypes. Thus, although TNF-α is one of the major substrates of TACE, our results strongly suggest that HNE-mediated GCM does not depend on TNF-α levels in vivo. Although somewhat surprising, the increased TNF-α levels in Tace-deficient mice could be explained by the activation of other enzymes capable of processing TNF-α processing, including MMP7, ADAM10, and ADAM19 (11). In particular, ADAM10 is known to

Fig. 4. Immunohistochemical staining of MUC5AC. Immunohistochemical staining of MUC5AC was performed to specifically immunolabel goblet cells (×200 magnification, A and B). Strong immunostaining was detected on the tissue from Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> mice (wild-type mice) that were exposed to HNE, whereas no staining was observed on the tissue from Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> mice (Tace conditional deletion mice) that were exposed to HNE. The dotted areas in A and B are magnified in C and D. Secondary antibody alone (E) was used to confirm the specificity of the MUC5AC antibody. Scale bars, 100 μm.

Fig. 5. Muc5ac mRNA expression, tissue inflammation index, and concentration of TNF-α in bronchoalveolar lavage (BAL) fluid. A: Muc5ac mRNA expression in homogenized lung tissue was determined by real-time PCR analysis and normalized to the expression of Gapdh. B: tissue inflammation, which was determined by hematoxylin and eosin staining, was not significantly different between the genotypes. Number of mice used for each condition: Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with PBS (wild-type-PBS), n = 6; Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with HNE (wild-type-HNE), n = 9; Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with PBS (Tace conditional deletion-PBS), n = 3; Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with HNE (Tace conditional deletion-HNE), n = 14. C: concentration of TNF-α in BAL fluid. Number of mice used for each condition: Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with PBS (wild-type-PBS), n = 2; Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with HNE (wild-type-HNE), n = 3; Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with PBS (Tace conditional deletion-PBS), n = 3; Tace<sup>fl<sup>ox/</sup>fox<sup>/Cre<sup>−/−</sup></sup> with HNE (Tace conditional deletion-HNE), n = 5.
function as a TNF-α sheddase when TACE is deficient in vivo and in vitro (17, 19).

Taken together, these results demonstrate that TACE, but not TNF-α, plays a necessary role in HNE-induced GCM. Previous evidence has linked GCM to EGFR activation (5, 16, 25) and TACE to shedding of bioactive EGFR ligands (2). Our results are consistent with conditional deletion of TACE attenuating GCM through inhibition of EGFR activation, which builds on evidence that EGFR is a prominent mediator in airway remodeling events, including GCM (3). TACE proteolytically sheds a variety of EGFR ligands, such as amphiregulin (18), TGf-α (14, 29), and heparin-binding epidermal growth factor-like growth factor (30), which together contribute to the activation of EGFR and the development of GCM in different model systems. We previously showed that TACE deletion attenuated EGFR-mediated responses in cultured mouse tracheal epithelial cells in vitro (30), demonstrating that TACE acts as a common upstream mediator of EGFR ligand shedding in these cells. Thus our results are consistent with a role of TACE in HNE-induced airway remodeling and suggest that further efforts should focus on the specific ligands that link TACE to GCM in vivo.

In conclusion, the conditional deletion of TACE by use of a Cre-loxP system significantly attenuated the number of goblet cells induced by HNE exposure, which demonstrates that TACE is a regulator of HNE-induced GCM in mice. This finding suggests that TACE could be a potential target in the treatment of GCM in patients with chronic airway diseases.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


