Differential expression of the glycosylated forms of MUC1 during lung development

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Human MUC1 mucin is a high-molecular weight transmembrane glycoprotein expressed on the apical surface of the simple epithelia of many different tissues. Previous investigations suggest the involvement of MUC1 in epithelial cytodifferentiation and glandular morphogenesis. However, the role of MUC1 in the development of the fetal respiratory tracts has so far been poorly investigated. To obtain more information on the roles of MUC1 during fetal lung development, we examined the expression and distribution of MUC1 by immunohistochemical staining of postmortem lung specimens from fetuses and neonates of various gestational ages. Three monoclonal antibodies, HMF1, HMF2, and anti-KL-6, which bind different glycosylation variants, were used. Each monoclonal antibody has been shown to recognize heavily-glycosylated MUC1, sparsely-glycosylated MUC1, and sialylated carbohydrate side chains of MUC1, respectively. At 13 weeks of gestation, the terminal respiratory tracts were diffusely stained with HMF1 and anti-KL-6. Sparsely-glycosylated MUC1, as recognized by HMFG2, was detected only in the distal portions of the terminal bronchioles that divided into respiratory bronchioles. As such development continued, MUC1, recognized by HMFG1 and anti-KL-6, was detected throughout the bronchioles and terminal sacs, although HMFG1 immunoreactivity decreased in intensity towards the terminal sacs. Sparsely-glycosylated MUC1, as recognized by HMFG2, was mainly observed in the terminal portions. In the adult lungs, both the alveolar spaces and the respiratory bronchioles stained with HMF1 and anti-KL-6. However, the distribution of sparsely-glycosylated MUC1 was limited in the alveolar epithelial cells. Our investigation demonstrated that variants of MUC1 were expressed in the fetal respiratory tracts as early as 13 weeks of gestation, and its expression persisted even after lung maturation. The precise roles of MUC1 were not determined in the present study; however, different glycosylation variants of MUC1 may be associated with the development of different regions of the terminal respiratory tract.

Key words: MUC1, glycosylation, sialic acid, lung development, respiratory tract.

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Paper accepted on February 22, 2007


Human MUC1 mucin is a high-molecular weight transmembrane glycoprotein, which is expressed on the apical surface of most simple epithelia, including that of the mammary gland, female reproductive tract, lung, kidney, stomach, gallbladder and pancreas. The extracellular domain of MUC1 consists of a variable number of amino acid tandem repeats, which include serine and threonine residues. These residues undergo tissue specific O-glycosylation to generate a broad range of glycosylated variants (Hudson et al., 2001; Brayman et al., 2004). The functions attributed to MUC1 include the lubrication and dehydration of cell surfaces, and protection from microorganisms and degradative enzymes. In addition, MUC1 is an effective inhibitor of both cell-cell and cell-extracellular matrix interactions in both normal and malignant tissues. In fact, glycosylated variants of MUC1 have been reported to correlate with both tumor progression and prognosis in a variety of carcinomas (Cao et al., 1997; Utsunomiya et al., 1998; Mommers et al., 1999; Tamada et al., 2002).

During human and mouse embryonic development, the expression of MUC1 and the mouse homologue Muc1 was found to coincide with epithelial sheet and glandular formation (Braga et al., 1992; Buisine et al., 1999). In human fetal lungs, MUC1 mRNAs were first detected in trachea, developing bronchi, and epithelial tubules between nine and ten weeks of gestation (WG), before the epithelium acquires any functional activities in the respiratory tracts. These observations suggest the involvement of MUC1 in epithelial cytodifferentiation and glandular morphogenesis (Chambers et al., 1994; Buisine et al., 1999). However, the role of MUC1 in fetal lung tissue requires further investigation.

The histogenesis of fetal and neonatal lungs can be divided into four distinct stages. The pseudoglandular stage lasts until 16 WG and it is characterized
by the formation of terminal bronchioles. The canalicual stage, in which terminal bronchioles divide into respiratory bronchioles, lasts from 16 to 28 WG. By 28 WG, respiratory bronchioles divide into several alveolar ducts, and thin-walled terminal sacs begin to develop. The saccular stage, which is characterized by the formation of terminal sacs (primitive alveoli), lasts from 28 to 36 WG, and a limited respiration is possible at this stage. The alveolar stage of lung histogenesis begins after 36 WG.

To obtain more information on the role of MUC1 during fetal lung development, we examined the expression and distribution of MUC1 by immunohistochemical staining of postmortem lung specimens from fetuses and neonates of various gestational ages. Three monoclonal antibodies (mAb) binding to different glycosylation variants, namely HMFG1, HMFG2, and anti-KL-6, were used. We also used an mAb of surfactant protein A (SP-A) as a marker of mature type II pneumocytes. In the present study, lung specimens from fetuses and neonates ranging from 13 to 41 WG were examined, thereby allowing us to assess the differential expression patterns of the glycosylated variants of MUC1 during all four stages of fetal and neonatal lung development.

Materials and Methods

All fetal and neonatal lung tissue specimens examined in this study were obtained from autopsy at the Department of Pathology, Tohoku University School of Medicine, Sendai, Japan. The cause of death of fetuses was either a spontaneous or therapeutic abortion, and the neonates died from non-pulmonary causes including congenital heart disease and/or cerebroventricular abnormalities. We also surveyed the lung specimens to confirm the absence of any pulmonary abnormalities by examining hematoxylin-eosin stained lung tissue specimens. Four adult lung specimens were prepared from the tumor free portion of the resected lungs from lung cancer patients at the Department of Molecular and Internal Medicine, Hiroshima University, Japan. The informed consent was obtained from either the patients or the families of the patients. Approval was obtained from the ethics committees of the School of Medicine, Tohoku University, and from the Graduate School of Biomedical Sciences, Hiroshima University.

Each specimen was fixed in 10% phosphate-buffered formalin, embedded in paraffin, and then was sectioned into 4 µm tissue sections for histological and immunohistochemical evaluation. Immunohistochemical analyses were performed using the avidin-biotin-peroxidase complex (ABC) method (Vectastain®, Vector Laboratories, USA). The tissue sections were first deparaffinized in xylene and rehydrated through a graded ethanol series. All sections, except for those subjected to staining with anti-KL-6, were then heated in a microwave in 0.01 M citrate buffer for antigen retrieval (Cattoretti et al., 1993). Next, all sections were sequentially incubated in 0.3% H2O2 in methanol for 30 min to block endogenous peroxidase activity. After being washed with phosphate-buffered saline, the sections were incubated with the mAbs against MUC1 (HMFG1, Ylem, Italy, 1:100; HMFG2, Ylem, 1:100; anti-KL-6, 3 µg/mL), surfactant protein A (SP-A) (PE10, DAKO, Denmark, 1:100) at 4°C overnight.

The epitope of HMFG1 is the MUC1 core peptide APDTR containing high molecular weight carbohydrate side chains, and this antibody detects heavily-glycosylated MUC1 (Burchell et al., 1983; Burchell et al., 1993; Price et al., 1998). The binding of HMFG2 to the MUC1 core peptide DTR is also influenced by the carbohydrate chains, and this antibody detects sparsely-glycosylated MUC1 (Burchell et al., 1993; Buem et al., 1999). Anti-KL-6 reacts with a sialylated carbohydrate antigen classified as Cluster 9 (MUC1 mucin) (Kohno et al., 1989; Stahel et al., 1994). After being washed thoroughly, the sections were treated with a biotinylated anti-mouse immunoglobulin antiserum, washed again and then were incubated with the ABC complex. Positive staining was visualized with 3, 3′-diaminobenzidine, and the sections were counterstained with hematoxylin.

Results

The results of immunohistochemical studies performed on lung tissue specimens from the fetuses and neonates of various gestational periods and adult patients using three anti-MUC1 mAbs (HMFG1, HMFG2, anti-KL-6) and anti-SP-A mAb (PE10) are summarized in Table 1. MUC1, recognized by HMFG1 and anti-KL-6, was found to be diffusely expressed in the epithelium of the bronchioles as early as at 13 WG (Figures 1A, C). In contrast, sparsely-glycosylated MUC1, recognized by HMFG2, was detected only in the distal portion of
the bronchioles (Figure 1B). SP-A was not observed in the respiratory tracts at 13 WG (Figure 1D). These staining patterns persisted throughout the pseudoglandular stage. During the canalicular stage, MUC1, recognized by HMFG1 and anti-KL-6, was detected in the epithelium of the terminal to respiratory bronchioles (Figures 2A, C). We also found the apical side of both ciliated and non-ciliated cells to be stained with these two antibodies. However, only the apical lumen of the distal portion of bronchioles was stained with HMFG2 (Figure 2B). SP-A expression was observed to be scattered in the epithelial lining cells of the terminal sacs (Figure 3D). In the alveolar stage, when the alveoli mature, strong positive staining with anti-KL-6 was observed in the alveolar and the bronchiolar epithelia (Figure 4C). MUC1, recognized by HMFG1, was abundantly expressed in the bronchioles but patchily detected in the alveoli (Figure 4A). On the other hand, MUC1, which was recognized by HMFG2, was positive in the alveoli, but not in the bronchioles, and these findings were similar to those for SP-A (Figure 4B, D).

In the adult lungs, the pattern of immunoreactivity with these four antibodies was identical to the staining pattern in the neonatal lungs of the alveolar stage. MUC1, recognized by anti-KL-6, was detected in the bronchiolar epithelium and type II

Table 1. Immunohistochemical findings regarding the expression of MUC1 antigens and SP-A.

<table>
<thead>
<tr>
<th>Ages (WG)</th>
<th>Number of samples</th>
<th>HMFG1</th>
<th>HMFG2</th>
<th>Anti-KL-6</th>
<th>PE10</th>
</tr>
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<tbody>
<tr>
<td>13</td>
<td>1 bronchioles</td>
<td>++</td>
<td>++d</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>3 bronchioles</td>
<td>++</td>
<td>++d</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>2 bronchioles</td>
<td>++</td>
<td>++d</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>6 bronchioles</td>
<td>++</td>
<td>++d</td>
<td>++</td>
<td>+/-</td>
</tr>
<tr>
<td>19</td>
<td>1 bronchioles</td>
<td>++</td>
<td>++d</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
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<td>2 bronchioles</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>3 bronchioles</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>2 bronchioles</td>
<td>++/++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>28</td>
<td>1 bronchioles</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>1 bronchioles</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
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<td>++</td>
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<td>3 bronchioles</td>
<td>++/+</td>
<td>+/-</td>
<td>++</td>
<td>+/-</td>
</tr>
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<td>5 bronchioles</td>
<td>++/+</td>
<td>-</td>
<td>++</td>
<td>+/-</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Adult</td>
<td>4 bronchioles</td>
<td>++</td>
<td>-</td>
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<td>+/-</td>
</tr>
<tr>
<td></td>
<td>alveoli</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
</tr>
</tbody>
</table>

-, absence; +, presence; ++, presence with high immunoreactivity; WG, weeks of gestation; d, stained only in the distal portion of the bronchioles.
Figure 1. Immunohistochemical staining of fetal lung at 13 weeks of gestation with HMFG1 (A), HMFG2 (B), anti-KL-6 (C), and PE10 (D). Heavily-glycosylated and sialylated MUC1, as recognized by HMFG1 and anti-KL-6, respectively, were observed in the apical lumen of the proximal to distal portions of the bronchioles (A and C). In contrast, sparsely-glycosylated MUC1, as recognized by HMFG2, was only detected on the apical lumen of the distal portion (Arrows) but not in the proximal portion (Pr) of terminal bronchioles (B). Immunostaining for SP-A was negative (D). Bar = 100 µm.

Figure 2. Immunohistochemical staining of the fetal lung at 18 weeks of gestation with HMFG1 (A), HMFG2 (B), anti-KL-6 (C), and PE10 (D). Heavily-glycosylated and sialylated MUC1, as recognized by HMFG1 and anti-KL-6, respectively, were detected in the apical lumen of proximal to distal portions of the bronchioles (A and C). In contrast, HMFG2 staining was observed in the distal portion of the terminal bronchioles (B). Immunostaining for SP-A was only scatteredly positive in the distal portion of the bronchioles (D). The arrows denote the distal portion of the bronchioles. Bar = 100 µm.
pneumocytes (Figure 5C). Both the bronchiolar epithelium and type II pneumocytes also stained with HMFG1. However, the staining was more intense in the bronchioles than in the alveoli (Figure 5A). MUC1, which was recognized by HMFG2, and SP-A were mainly positive in the type II pneumocytes, but they were either negative or very weakly positive in the bronchioles (Figures 5B, D).

Discussion

In the present study, we have demonstrated that MUC1 mucin is expressed diffusely in the fetal respiratory tracts at 13 WG and throughout development, and is detected throughout the terminal and respiratory bronchioles and the terminal sacs. This finding is consistent with previous observations demonstrating that MUC1 mRNAs are detected in the fetal airway from 9 WG. This implies an association of MUC1 with lung morphogenesis (Buisine et al., 1999).

In addition, we demonstrated the presence of different glycosylation variants of MUC1 in the terminal respiratory tracts of fetal, neonatal, and adult lungs. MUC1 core protein generally undergoes tissue specific O-glycosylation to generate a broad range of carbohydrate chain variants. The specificity of the MUC1 antibodies is generated by the difference in glycosylation levels of the carbohydrate side chains. Interestingly, sparsely-glycosylated MUC1, recognized by HMFG2, was observed only in the distal portions of the bronchioles during the pseudoglandular and canalicular stages. At the end of the canalicular stage, when the terminal sacs (primitive alveoli) began to develop, sparsely-glycosylated MUC1 was only positive in the epithelium of the terminal sacs and alveoli, but not in the bronchioles. In contrast, heavily-glycosylated MUC1, recognized by HMFG1, was detected throughout the terminal respiratory tracts at 13 WG, and this trend persisted during the pseudoglandular and canalicular stages. At the end of the canalicular stage, the expression of heavily-glycosylated MUC1 became weak in the terminal sacs and alveoli.

Lung development involves epithelial cell proliferation and migration, which are similar cellular behaviors to those which modulate malignant tissue invasion in cancer (Coraux et al., 2002). In cancer cells, sparsely-glycosylated MUC1 is generally
Figure 4. Immunohistochemical staining of neonatal lung at 38 weeks of gestation with HMFG1 (A), HMFG2 (B), anti-KL-6 (C), and PE10 (D). Heavily-glycosylated and sialylated MUC1, recognized by HMFG1 and anti-KL-6, respectively, were observed in both the apical lumen of the bronchioles (Br) and alveoli (**). However, immunoreactivity with anti-KL-6 in the alveoli was stronger in comparison with that of HMFG1 (A and C). Immunoreactivity with HMFG2 was positive in the alveoli but negative in the bronchioles (B). Immunostaining for SP-A was positive in the alveolar epithelium (D). Br denotes the bronchioles, and ** indicates the alveoli. Bar = 100 μm.

Figure 5. Immunohistochemical staining of the adult lung with HMFG1 (A), HMFG2 (B), anti-KL-6 (C), and PE10 (D). Immunoreactivity with HMFG1 was observed in the apical lumen of bronchioles and faintly in type II pneumocytes (A). Sialylated MUC1, recognized by anti-KL-6, was positive in both the apical lumen of bronchioles and the type II pneumocytes (B). In contrast, under-glycosylated MUC1, recognized by HMFG2, was detected only in the type II pneumocytes of alveoli but negative or weakly positive in the epithelium of bronchioles (B). Immunostaining for SP-A was only positive in the alveolar epithelium (D). The arrows denote the type II pneumocytes of the alveoli and Br indicates the bronchioles. Bar = 100 μm.
expressed more than heavily-glycosylated MUC1. In fact, previous studies have shown that sparsely-glycosylated MUC1 is more abundant in breast cancer tissue than heavily-glycosylated MUC1. The opposite is true in normal breast tissue (Hull et al., 1989; Lloyd et al., 1996). The specific localization of sparsely-glycosylated MUC1 in the very distal portions of the terminal respiratory tracts, sites of bronchiolar and alveolar development, suggests an association of sparsely-glycosylated MUC1 in lung branching morphogenesis.

Another interesting finding in the present study is that MUC1 with sialylated carbohydrate side chains, recognized by anti-KL-6, was expressed throughout the epithelium of the terminal respiratory tracts from 13 WG and postnatally. This finding is consistent with previous observations which demonstrated an increase in sialic acid expression in lung epithelial structures from 15 WG (Cerna et al., 2002). One previous study has demonstrated the expression of KL-6 in premature lung tissue as early as 23 WG (Sun et al., 2003), however, our finding revealed that its expression in the fetal lung starts at a much earlier gestational age. Sialic acid is a 9-carbon monosaccharide, usually located at the outermost position of the carbohydrate side chains of glycoconjugates, and possesses a strong negative charge. Although sialic acid is involved in cell attachment through binding Siglecs mainly expressed in immune cells (Varki et al., 2006), it is believed to contribute to repulsion between epithelial cells and to the extracellular matrix (Kohno et al., 1989; Narayanan, 1994; Varki, 1997; Matsukita et al., 2003). In addition to the anti-adhesive function of MUC1 itself, the expression of sialylated MUC1 throughout the luminal surface of the terminal respiratory tracts during lung histogenesis suggests an important role of sialylated MUC1 in the formation and maintenance of the luminal and complex branching structure by an anti-adhesive and repulsive property.

In conclusion, this study demonstrated the differential expression patterns of glycosylation variants of MUC1 during human lung histogenesis, more specifically in the terminal respiratory tracts as early as 13 WG. The detection of sparsely-glycosylated MUC1 only in the very distal portion of the respiratory tracts suggests its possible association in lung branching morphogenesis and suggests a role in epithelial cell proliferation and migration. The expression of heavily-glycosylated and/or sialylated MUC1 throughout the terminal respiratory tract epithelium suggests a role in maintaining a luminal architecture through their anti-adhesive and repulsive properties. Although our findings suggest a strong association of MUC1 in the morphogenesis of the terminal respiratory tracts, the precise roles of MUC1 in fetal lungs remain undetermined.

References


Lloyd KD, Burchell J, Kudryashov V, Yin BW, Taylor-Papadimitriou J. Comparison Of O-Linked Carbohydrate Chains In Muc-1 Mucin From Normal Breast Epithelial Cell Lines And Breast Carcinoma Cell Lines. Demonstration Of Simpler And Fewer Glycan Chains In
Varki A, Angata T, Siglec--The Major Subfamily Of I-Type Lectins. Glycobiology 2006; 16; 1r=27r.