MUC1 Is A Novel Marker for the Type II Pneumocyte Lineage during Lung Carcinogenesis

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ABSTRACT

Abnormalities in mucin-type glycoprotein expression have been documented in a variety of cancers, identifying these molecules as targets for immunologically based therapies and prognostic/diagnostic assays. We examined the expression of the membrane-bound MUC1 mucin in normal, histologically atypical, and neoplastic lung to determine its potential contribution to lung carcinogenesis. In vivo, intense MUC1 immunoreactivity was present in normal type II pneumocytes as well as in a range of atypical lesions derived from type II cells and >60% of primary and metastatic non-small cell lung cancers. Expression was not associated with altered survival, although it was highly correlated with the adenocarcinoma histological subtype and carcinogenesis model. A correlation model using 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone-exposed hamsters revealed that MUC1 mRNA increased prior to the histological appearance of tumors. In vitro studies using MUC1 expressing non-small cell lung cancer cell lines revealed that differentiation away from a type II cell lineage was associated with dramatic down-regulation of MUC1. We propose that MUC1 is a powerful new marker for the type II pneumocyte cell lineage that allows us to follow the type II pneumocyte lineage during the process of lung carcinogenesis.

INTRODUCTION

The mucin family of glycoproteins contributes to the protective and lubricating functions of mammalian mucus by imparting properties of viscoelasticity and aggregation (1). These proteins are characterized by a variable number of amino acid tandem repeats and extensive O-glycosylation at serine and threonine residues, resulting in a composition of 50–80% carbohydrate by weight. To date, at least eight mucin genes, encoding the protein backbone of these glycoproteins, have been identified and at least partially sequenced. Of these genes, MUC1 codes for the only membrane-bound mucin (1–4). Unlike other mucins, MUC1 has a large extracellular domain along with transmembrane and cytoplasmic domains (2, 4).

In normal tissues, MUC1 is expressed in the apical plasma membrane of the epithelial component of many organs including breast, salivary gland, esophagus, stomach, pancreas, bile ducts, lung, kidney, bladder, uterus, and rete testes (5–11). In the corresponding carcinomas, MUC1 has frequently been found to have increased, unpolarized expression as well as altered glycosylation, allowing for greater immunogenicity by revealing otherwise masked epitopes in its protein core (5, 10–17). In the lung, MUC1 mRNA is abundantly expressed in total lung extracts, and increased expression has been found in cancers, particularly in well-differentiated adenocarcinomas (5, 6, 10–12, 16). MUC1 protein expression has been reported in the apical membranes of normal bronchial epithelial cells and in submucosal serous glands, whereas the alveolar epithelium has been described as negative or trace positive (5, 10). However, no comprehensive studies of MUC1 protein expression in normal and neoplastic lung have been reported to date.

Several studies have suggested that MUC1 may facilitate epithelial carcinogenesis. In an immunohistochemical study of ovarian carcinomas, Dong et al. (18) found that high levels of MUC1 correlated with greater invasiveness. Cell lines transfected with MUC1 cDNA have reduced cell-matrix and cell-cell adhesion, which may account for greater metastatic ability (19–21). MUC1-transfected melanoma cells are not able to form conjugates with lymphokine-activated killer cells and CTLs (20), and purified MUC1 protein reversibly inhibits proliferation of cultured T cells (22). MUC1 has also been shown to form complexes with Grb2 and Sos through its cytoplasmic tail, suggesting a potential role in signal transduction (23). Thus, MUC1 may contribute to carcinogenic progression through modulation of cell adhesion, the immune system, and cell signaling.

Up-regulated expression of MUC1 in carcinomas has pointed to possible uses in prognosis, immunotherapy, and gene therapy. Assays for CA 15-3, which recognize MUC1, have been used to monitor early breast cancer (24–26), and given the aberrant expression of MUC1 in many cancers, several recent studies have focused on MUC1 as a target for vaccine development (27–29). Data from Mensdorff-Pouilly et al. (27) suggest that a humoral immune response to MUC1 protects against disease progression in mammary carcinogenesis. In addition, Chen et al. (30) have used the MUC1 promoter for adenoviral delivery of therapeutic genes to cancer cells.

Given the widespread distribution of MUC1 in epithelial tissues and its potential contribution to carcinogenic progression, we examined the role of MUC1 in lung carcinogenesis in the present study. We report for the first time the presence of MUC1 in normal type II pneumocytes in the alveolar epithelium as well as in atypical and neoplastic lesions derived thereof. Type II pneumocytes, identified by their distinctive morphology and production of surfactant-associated proteins such as SP-A, are thought to function as progenitor cells for normal and neoplastic epithelium during the repair of injury and during carcinogenesis (31–34). Thus, expression of MUC1 appears to be preserved during the process of lung carcinogenesis. Cell line studies indicated that induced differentiation and reversion to a more normal phenotype via pharmacological manipulation was accompanied by decreased MUC1 mRNA and protein expression. These results suggest that MUC1 is a novel and powerful marker for the alveolar type II pneumocyte lineage, and its loss is associated with differentiation away from this lineage.

MATERIALS AND METHODS

Immunohistochemistry. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissues using citrate-microwave antigen retrieval as described previously (35). The NCI-MUC1-CORE antibody was used for all studies (clone Ma552; Novocastra, Burlingame, CA). This is a mouse monoclonal antibody directed at a hexapeptide in the tandem repeat region in...
the protein core of MUC1. In addition, 20 specimens were also stained with a second mouse monoclonal antibody, NCL-MUC1 (clone Ma695; Novocastra), which recognizes a carbohydrate epitope of the MUC1 glycoprotein. A third antibody (MUC1, clone HPMVF; Pharmingen, San Diego, CA), also directed at the tandem repeat region in the core peptide of MUC1, was also used in four specimens to confirm the staining patterns. Immunohistochemistry was performed using a modified avidin-biotinylated peroxidase technique using Vectastain kits from Vector Laboratories (Burlingame, CA; Ref. 35).

Results were evaluated independently by three observers (J. J., E. S., and R. I. L.) without prior knowledge of the patients’ clinical histories. The tumors were scored using the following scale: 0, no positive cells; 1, <1% tumor cells positive; 2, ≥1% and <10% tumor cells positive; 3, ≥10% and <50% tumor cells positive; 4, ≥50% and <75% tumor cells positive; and 5, ≥75% tumor cells positive. Intensity of the staining was scored on a scale of 0 to + (weak) to + + + (strong). Tumor specimens containing ≥10% cells with MUC1 immunoreactivity, regardless of intensity (score 3–5), were considered positive for use in clinicopathological correlation. Atypical regions containing four or more contiguous positively staining cells were considered positive.

**Patient Specimens.** Surgical sections of NSCLC tumors (49 primary lung, 33 lymph node metastases, and 10 other metastases) from 92 patients with available clinical follow-up were examined by immunohistochemistry. These specimens were collected as part of a prospective clinical trial of patients with any stage or histological subtype of NSCLC who were entered into the study at the NCI-Navy Medical Oncology Branch between May 1984 and August 1990 (36). Each specimen was fixed in 10% formalin and embedded in paraffin. The characteristics of these patients are shown in Table 1. In addition, 80 specimens from patients with NSCLC obtained from Johns Hopkins University (37) were also stained. Clinical correlation was not available for these patients.

To examine MUC1 expression in normal and atypical lung, surrounding lung obtained at the time of resection for lung cancer from 17 patients at Johns Hopkins University (37) and lungs from two accident victims without a cancer diagnosis (obtained through National Disease Research Interchange, Philadelphia, PA) were also studied. These specimens were also fixed in formalin and embedded in paraffin.

**Clinicopathological Analysis.** Clinicopathological analysis was performed for the 92 NSCLC specimens from the patients described in Table 1. The patients were divided into two groups at initial diagnosis for treatment purposes. Patients with stage I, II, or IIIA tumors were grouped as potentially curative, whereas patients with stage IIIB or IV tumors comprised the palliative group. Survival time was defined as the time period from the date of diagnosis until death or last follow-up. Mantel's test for trend was used to compare survival time between groups.

**RESULTS**

**MUC1 Expression in Primary and Metastatic Neoplastic Lung.** Immunohistochemical analysis of MUC1 protein expression was carried out on 92 formalin-fixed, paraffin-embedded, surgical specimens of NSCLC tumors from patients with available clinical follow-up and on 80 specimens from patients without clinical follow-up. All cases were studied with NCL-MUC1-CORE. MUC1 immunoreactivity was observed in 62% of the 172 tumors, with typical positively and negatively stained cases shown in Fig. 1a and b. Three distinct staining patterns were noted in tumors. Membranous staining (Fig. 1c), along the lumen showing the apical polarity previously described for MUC1 expression in normal tissues (1, 5), was seen primarily in papillary adenocarcinomas. Diffuse cytoplasmic staining demonstrating a loss of normal apical polarity (Fig. 1: a, low power view, and d, high power view) was frequently present. In addition, intense focal staining was seen in some cancers as a globular staining pattern (Fig. 1e). In some cases, more than one staining pattern was seen in different areas. A subset of patients was studied with two additional anti-MUC1 antibodies. Immunoreactivity was slightly more intense, and a greater percentage of cells was positive with the NCL-MUC1 antibody (recognizing a carbohydrate epitope of MUC1) than with NCL-MUC1-CORE (which recognizes the tandem repeat). However, of 20 cases examined with both of these antibodies, no case that was classified as positive or negative by one antibody was reclassified after staining with the second antibody. In addition, four cases, including two

Table 1 MUC1 immunoreactivity in 92 NSCLC patients with clinical follow-up

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive</th>
<th>Negative</th>
<th>P2</th>
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<tbody>
<tr>
<td>No. of cases (%)</td>
<td>59 (64%)</td>
<td>33 (36%)</td>
<td>0.51</td>
</tr>
<tr>
<td>Age (yr) median</td>
<td>55.5</td>
<td>57.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>35/23</td>
<td>26/8</td>
<td>0.16</td>
</tr>
<tr>
<td>Smoking (pack-yr), median</td>
<td>40.0</td>
<td>50.0</td>
<td>0.59</td>
</tr>
<tr>
<td>Cell line development</td>
<td>13/58</td>
<td>63/4</td>
<td>0.46</td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td>I-IIIA</td>
<td>33</td>
<td>22</td>
</tr>
<tr>
<td>I-IVB</td>
<td>25</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td>Adenocarcinoma</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td>Others</td>
<td>14</td>
<td>12</td>
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* Positive, ≥10% tumor cells positive for MUC1.
MUCl Expression Increases during NNK-induced Hamster Carcinogenesis. Evidence for the increased expression of MUCl mRNA during lung carcinogenesis was found in an in vivo hamster carcinogenesis model. Hamsters were treated with the tobacco-specific nitrosamine, NNK, for up to 24 weeks, by which time lung tumors developed in 100% of the animals. They were sacrificed after treatments of varying lengths, with some animals being allowed to recover for 6 weeks before sacrifice. Total lung RNA was isolated, and MUCl expression was examined by Northern blot analysis. Fig. 5 shows that abundant MUCl expression was present in the normal (vehicle-treated) hamster lung, but expression increased as early as 6 weeks after beginning NNK treatment. This increase occurred before tumors were observed in the lung. Furthermore, MUCl mRNA did not diminish to baseline, even if the animals were allowed to recover for an additional 6 weeks.

MUCl Expression in Normal and Atypical Human Lung. To determine MUCl expression in normal lung, we analyzed histologically normal regions from the surrounding lung of 30 patients with NSCLC, as well as lungs from 2 patients who did not have lung cancer. The conducting airways were generally negative for MUCl (Fig. 3b) with 16 of 24 histologically normal bronchi showing no immunoreactivity. Some staining of basal cells was present in 5 of 24 cases, whereas traces of membranous staining at the lumenal border were seen in 3 cases. The bronchioles exhibited a similar pattern, with occasional staining of basal cells. In addition, membranous immunoreactivity was noted in clusters of nonciliated cells in the terminal bronchioles in five cases. In agreement with previous studies (5, 10), mucinous bronchial submucosal glands were negative in 16 of 16 cases, whereas serous glands showed low level of expression in some cases (Fig. 3d). In the alveolar epithelium, histologically normal type II pneumocytes were positive in every case, whereas type 1 cells were negative (Fig. 3f). This pattern of staining was confirmed using two additional anti-MUCl antibodies.

MUCl expression in atypical lesions from the surrounding lung is summarized in Table 2 and pictured in Fig. 4. Atypical lesions in the conducting airway were all negative for MUCl, including six goblet cell hyperplasias (Fig. 4b), four squamous metaplasias, four bronchial dysplasias (Fig. 4d), and four carcinomas in situ. In contrast, atypical lesions of the alveolar epithelium expressed MUCl at high levels. Every case of type II cell hyperplasia (16 cases; Fig. 4f), atypical type II cells (four cases), atypical alveolar hyperplasia (three cases; Fig. 4h), and alveolar carcinoma in situ (one case) was immunoreactive for MUCl. In contrast to neoplastic cells, the staining in both atypical and normal lung was solely membranous, demonstrating that the MUCl polarity of normal lung was preserved in the atypical phenotype.

Clinicopathological correlation was determined for the 92 patient cohort with available clinical follow-up. This cohort consisted of 49 primary lung specimens, 33 lymph node metastases, and 10 distant metastases and represented a broad range of stages and histologies as summarized in Table 1. MUCl expression was not correlated with age, sex, smoking history, ability to generate a cell line from the primary tumor, or stage, although it was found to be highly associated with the adenocarcinoma histology (P < 0.001). No association existed between MUCl expression and immunoreactivity for the tumor suppressor p53 or proliferating cell nuclear antigen, which have been studied previously in this cohort of patients (46). No correlation with survival was found in either the group treated with curative intent (Fig. 2a) or with palliative intent (Fig. 2b) when comparing MUCl-negative (<10% positive cells) versus MUCl-positive (≥10% positive cells) tumors.

MUCl expression in atypical lesions from the surrounding lung is summarized in Table 2 and pictured in Fig. 4. Atypical lesions in the conducting airway were all negative for MUCl, including six goblet cell hyperplasias (Fig. 4b), four squamous metaplasias, four bronchial dysplasias (Fig. 4d), and four carcinomas in situ. In contrast, atypical lesions of the alveolar epithelium expressed MUCl at high levels. Every case of type II cell hyperplasia (16 cases; Fig. 4f), atypical type II cells (four cases), atypical alveolar hyperplasia (three cases; Fig. 4h), and alveolar carcinoma in situ (one case) was immunoreactive for MUCl. In contrast to neoplastic cells, the staining in both atypical and normal lung was solely membranous, demonstrating that the MUCl polarity of normal lung was preserved in the atypical phenotype.
Table 2  MUC1 immunoreactivity in atypical lesions of nonneoplastic lung

<table>
<thead>
<tr>
<th>Lesion</th>
<th>MUC1 Immunoreactivity</th>
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<tbody>
<tr>
<td>Conducting airway</td>
<td>0/6 positive</td>
</tr>
<tr>
<td>Goblet cell hyperplasia</td>
<td>0/4 positive</td>
</tr>
<tr>
<td>Squamous cell metaplasia</td>
<td>0/4 positive</td>
</tr>
<tr>
<td>Bronchial dysplasia</td>
<td>0/4 positive</td>
</tr>
<tr>
<td>Squamous carcinoma in situ</td>
<td>0/4 positive</td>
</tr>
<tr>
<td>Alveolar epithelium</td>
<td>0/6 positive</td>
</tr>
<tr>
<td>Type 2 cell hyperplasia</td>
<td>16/16 positive</td>
</tr>
<tr>
<td>Atypical type 2 cells</td>
<td>4/4 positive</td>
</tr>
<tr>
<td>Atypical alveolar hyperplasia</td>
<td>3/3 positive</td>
</tr>
<tr>
<td>Alveolar carcinoma in situ</td>
<td>1/1 positive</td>
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Fig. 3. Photomicrographs of MUC1 expression in histologically normal lung tissues. a, normal bronchial mucosa; Lu, lumen (H&E stain, ×100). b, normal bronchial mucosa stained with MUC1 antibody demonstrates no immunoreactivity in epithelial cells (immunoperoxidase, ×100). c, normal bronchial submucosal glands. M, mucinous glands; S, serous glands (H&E stain, ×100). d, MUC1 immunoreactivity in normal bronchial submucosal glands is present in serous (S) but not mucinous (M) glands (immunoperoxidase, ×100). e, normal alveoli (H&E stain, ×100). f, normal alveoli show MUC1 immunoreactivity in scattered cuboidal type II cells but not type I cells (immunoperoxidase, ×100).

MUC1 Expression in NSCLC Cell Lines. To determine the expression of MUC1 in NSCLC, RNA was prepared for 10 NSCLC cell lines of varying histologies and one immortalized bronchial epithelial cell line. Five of the 10 NSCLC cell lines expressed MUC1 mRNA to varying degrees, whereas the immortalized bronchial epithelial cell line did not express MUC1 (Fig. 6). Of these five cell lines, four were established from patients with adenocarcinomas or bronchialalveolar carcinomas, whereas one (NCI-H1334) was derived from a patient with large cell carcinoma. Of note, NCI-H1334 has been found to express markers of peripheral lung origin including the Clara cell protein CC10 and SP-A by in situ hybridization (47).

Down-Regulation of MUC1 during Differentiation Induced by Tumor Promoters and Histone Deacetylase Inhibitors. We have shown previously that the NSCLC cell line NCI-H338 differentiates along a mucinous lineage after treatment with the tumor promoter mezerein, as determined by morphological changes, development of periodic acid-Schiff positivity, and growth arrest (48). MUC1 mRNA was markedly down-regulated during mezerein-induced differentiation in this cell line (Fig. 7A). Similarly, MUC1 mRNA was also markedly reduced after mezerein treatment in NCI-H441 (Fig. 7B), accompanied by decreased MUC1 protein expression as demonstrated by loss of immunoreactivity (results not shown). NCI-H441 expresses SP-A, a product of normal type II pneumocytes. Mezerein treatment resulted in down-regulation of SP-A, suggesting that these cells were differentiating away from a type II pneumocyte lineage.

In contrast, mezerein treatment did not decrease MUC1 mRNA in NCI-H332 (Fig. 7C) or NCI-H1944 (results not shown). Whereas mezerein slowed the growth of these two cell lines, it did not completely suppress growth or alter morphology (Fig. 7D and results not shown). In addition, gelsolin, an actin-regulatory protein the expression of which is decreased during lung carcinogenesis and that has been suggested to be a marker of differentiation (49), was down-regulated by mezerein in NCI-H332 but up-regulated in NCI-H441 (Fig. 7E). These data suggest that differentiation was not induced by mezerein in NCI-H332, and that down-regulation of MUC1 correlates with differentiation.

Alterations in chromatin structure through pharmacological inhibition of histone deacetylases have also been associated with changes in morphology suggestive of differentiation in cancer cell lines (49). Treatment of NCI-H441 and NCI-H332 with the histone deacetylase inhibitor trichostatin A resulted in a substantial decrease in MUC1 mRNA expression within 24 h (Fig. 7, B and C), correlating with reduced cell growth and morphological changes (results not shown). As with mezerein, trichostatin A treatment of NCI-H441 resulted in down-regulation of SP-A, once again suggesting differentiation away from a type II pneumocyte lineage (Fig. 7B). In addition, gelsolin was up-regulated by trichostatin A in both of these cell lines (Fig. 7E).
Thus, reversion to a nonproliferative phenotype was accompanied by reduced MUC1 expression.

DISCUSSION

This study addressed the potential role of MUC1 in pulmonary carcinogenesis through a multifaceted approach consisting of analysis of normal, histologically atypical, and neoplastic primary lung tissues as well as in vitro studies. The following novel findings have emerged from this analysis: (a) MUC1 is highly expressed in histologically normal type II pneumocytes and lesions derived thereof, as well as in cell lines demonstrating peripheral airway cell differentiation. We propose that MUC1 is a powerful novel marker for the type II pneumocyte lineage during carcinogenesis; (b) no correlation was found between MUC1 immunoreactivity and survival, despite prior suggestions that MUC1 may be a good prognostic marker in lung carcinogenesis (50). MUC1 expression was, however, strongly associated with the adenocarcinoma histology; and (c) chemically induced differentiation was accompanied by down-regulation of MUC1, suggesting that loss of MUC1 may serve as an indicator of a less malignant phenotype.

Although several previous studies have examined MUC1 expression in normal and neoplastic lung (5, 6, 10–12, 16), few studies have localized its expression to discrete lung cell types or examined atypical and potentially preneoplastic lesions. We therefore performed immunohistochemical analysis on normal, atypical, and neoplastic lung using three different antibodies, all of which gave similar results. Striking MUC1 immunoreactivity was found in histologically normal type II pneumocytes from patients with cancer as well as patients without a cancer diagnosis. Lesions derived from type II pneumocytes, such as type II cell hyperplasia, dysplastic type II cells, and atypical alveolar hyperplasia, were all consistently strongly positive. In contrast, immunoreactivity was not consistently found in bronchial or bronchiolar epithelial cells. MUC1 immunoreactivity was sometimes (but not always) found in the serous component of bronchial submucosal glands but never in the mucinous bronchial submucosal glands.

Our results differ from data reported by two prior studies that found MUC1 expression in bronchial epithelial cells using anti-MUC1 antibodies 139H2 and DF3 (5, 10). These studies also reported no (5) or trace (10) alveolar positivity using the 139H2 antibody. The most likely reason for differences in expression patterns between our study and the previous ones relates to the use of different antibodies.

Although all of these antibodies recognize MUC1, differences in glycosylation status are known to mask antigenic sites, and the ability of different antibodies to detect these alternative forms of MUC1 varies (51). The consistent results obtained by using three different antibodies in our study, and the presence of MUC1 in type II pneumocytes as well as all lesions derived from type II pneumocytes strongly suggests that MUC1 is, indeed, highly expressed in the alveolar epithelium. Furthermore, the coexpression of MUC1 and the type II cell marker SP-A in the cell line NCI-H441, together with their coordinated loss during induced differentiation by both mezerein and trichostatin A, adds support to the concept that MUC1 is a marker of the type II pneumocyte lineage.

Type II pneumocytes are thought to function as progenitor cells for the normal alveolar epithelium during repair of injury (31, 32). In conjunction with bronchiolar nonciliated secretory (Clara) and mucus-containing cells, type II cells are also thought to be the cells of origin of peripheral adenocarcinomas in humans and mice (32–34). Approximately 30–50% of adenocarcinomas express SP-A, the surfactant apoprotein produced in high quantity by normal type II pneumocytes (34, 52). In our study as well as in previous ones, the association between adenocarcinoma histology and MUC1 expression was very strong (5, 10, 11). This suggests that MUC1 expression is associated with a type II pneumocyte lineage in both neoplastic and nonneoplastic lung. Unlike CC10, a marker for Clara cell differentiation, MUC1 expression appears to be preserved during carcinogenesis, with MUC1 being highly expressed in normal type II cells as well as atypical lesions and overtly cancerous cells.

Despite the potential role of MUC1 in cancer progression, conflicting data exist concerning the link between MUC1 and cell growth and differentiation. Muc-1 null mice have decreased rates of tumor growth but no change in primary tumor formation compared with wild-type mice (53). Park et al. (43) have demonstrated that MUC1 expression in hamster tracheal surface epithelial cells is 6-fold higher in confluent cells than in actively growing cells, whereas in an endometrial adenocarcinoma cell line, MUC1 was most present in a proliferative phase (54). Lesuffleur et al. (55) observed high levels of MUC1 mRNA that remained almost constant during cell growth in mucous-producing differentiated HT-29 colon cancer cells. Correlations between MUC1 and mucous differentiation in the airway (56) and pseudopyhal differentiation in yeast (57) have also been demonstrated. Taken together, these studies suggest that MUC1 expression is not directly associated with cell growth per se but may be associated with differentiation along particular pathways in different cell types.

In our study, MUC1 expression decreased during differentiation induced by the tumor promoters mezerein and TPA (results not shown) and the histone deacetylase inhibitor trichostatin A in the NSCLC cell line NCI-H441. This correlated with down-regulation of
Fig. 7. Down-regulation of MUC1 mRNA expression during differentiation in NSCLC cell lines. Total cellular RNA was isolated after treatment with the tumor promoter mezerein or the histone deacetylase inhibitor trichostatin A, and after Northern blot transfer, sequential hybridization with 32P-labeled MUC1 or SP-A cDNA was performed (A–C). Ethidium bromide shadowing revealed equal RNA loading in all lanes in each experiment. MEZ, mezerein; TSA, trichostatin A. A. MUC1 mRNA during mezerein induced differentiation in NCI-H358. Down-regulation of MUC1 occurred within 72 h of mezerein treatment. B. MUC1 mRNA during mezerein- or trichostatin A-induced differentiation in NCI-H441. Down-regulation occurred within 24 h of treatment with both agents. SP-A expression was down-regulated in parallel. C. MUC1 mRNA during treatment with mezerein or trichostatin A in NCI-H322. Down-regulation occurred only after trichostatin A treatment. D. Growth curves after treatment with mezerein. Significant inhibition of cell growth was seen in NCI-H441 but not NCI-H322. Bars, SD. E. Gelsolin expression after treatment with mezerein or trichostatin A. Induction of gelsolin correlated with inhibition of cell growth, with induction occurring after both mezerein or trichostatin A treatment in NCI-H441 but only after trichostatin A treatment in NCI-H322.

SP-A, growth arrest, and up-regulation of gelsolin, an actin regulatory protein the expression of which is decreased during lung carcinogenesis (58) and that has been proposed to be a marker of the differentiated state (49). Thus, MUC1 decreased in parallel with differentiation away from a type II pneumocyte lineage. Curiously, mezerein and TPA (results not shown) did not down-regulate MUC1 in NCI-H322, although trichostatin A had this effect. This correlated with lack of induction of morphological changes and growth arrest suggestive of differentiation by the tumor promoters in NCI-H322, whereas trichostatin A induced growth arrest. As expected, gelsolin expression was induced in NCI-H322 by mezerein but not trichostatin A. Taken together, these data suggest that agents that induce differentiation away from a type II cell lineage and toward a nonproliferative phenotype down-regulate MUC1. These results are in contrast to studies by Percy et al. (59), who showed that induced differentiation in breast cancer cells by TPA and the histone deacetylase inhibitor sodium butyrate resulted in increased expression of MUC1. Most likely the effects of differentiating agents on MUC1 are context specific, and whether MUC1 increases or decreases depends on the pathway of differentiation and cell type.

Given the recent increase in pulmonary adenocarcinomas in the United States (60) without a concomitant reduction in mortality from
REFERENCES

1. MUCI IN LUNG CARCINOCENESIS

these tumors, there exists a need for better markers for identifying adenocarcinomas at an earlier, curable stage. Our results show that MUC1 does not appear to be an effective prognostic marker in vivo, because increased expression in lung cancer did not correlate with survival in our cohort of 92 patients with available clinical follow-up. However, the total amount of MUC1 appears to increase during carcinogenesis, as suggested by increased mRNA expression in NKK-treated hamsters that develop tumors. This may be due to either an increase in the number of MUC1 producing cells (such as seen in type II cell hyperplasia or atypical alveolar hyperplasia) or increased expression of MUC1 by cells that normally express it. Because MUC1 can be shed into blood and bodily fluids (such as bronchoalveolar lavage fluid or sputum), the increased total body burden of MUC1 could potentially be useful for monitoring disease relapse or perhaps even in early diagnosis of lung adenocarcinomas. Future studies will be needed to address this point.

In summary, the results of this study add new insight to our understanding of the expression and potential role of MUC1 in lung carcinogenesis. MUC1 was found to be frequently expressed in cancerous lung tissues, particularly of the adenocarcinoma histology, although its presence was not associated with altered survival. Immunoreactivity in adenocarcinomas was most likely associated with the type II pneumocyte differentiation pathway in these tumors because MUC1 was also highly expressed in histologically normal type II cells as well as in atypical lesions derived from type II cells. This indicates that MUC1 expression is preserved during cancer progression. During carcinogenesis, total lung MUC1 was found to increase in a hamster carcinogenesis model. Conversely, pharmacologically induced differentiation of NSCLC cell lines away from the progenitor type II pneumocyte lineage and toward a nonproliferative, less malignant state was accompanied by down-regulation of MUC1. These results suggest that MUC1 is a powerful new marker of the type II pneumocyte lineage and that the loss of MUC1 accompanies the reversion of cancerous tissues to a nonproliferative state. Use of this marker will facilitate research into differentiation-based therapies of lung cancer.

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