Prognostic Significance of MUC-1 Expression in Systemic Anaplastic Large Cell Lymphoma

George Z. Rassidakis, Andre Goy, L. Jeffrey Medeiros, Yunfang Jiang, Athanasios Thomaides, Yvonne Remache, Fernando Cabanillas, Andreas H. Sarris, and Frederic Gilles


ABSTRACT

Systemic anaplastic large cell lymphoma (ALCL) frequently carries the t(2;5)(p23;q35) and overexpresses anaplastic lymphoma kinase (ALK). MUC-1, a highly glycosylated transmembrane protein, is detected in normal and malignant epithelial cells and has been associated with a poorer patient survival in various human malignancies. We have shown previously that MUC-1 is expressed as a consequence of t(11;14)(q21;32) in a subset of diffuse large B-cell lymphomas. ALCLs are known to express MUC-1, but its clinical significance is undefined. For this study, eligible patients with ALCL were HIV negative, received anthracycline-containing regimens, and had pretreatment archival tissue. Expression of MUC-1 and ALK was determined immunohistochemically after heat-induced antigen retrieval. A 10% cutoff for MUC-1 positivity was used. We identified 63 patients with systemic ALCL (22 ALK+, 41 ALK−) with a median age of 47 years, and 41 were male. MUC-1 was shown previously that MUC-1 is expressed as a consequence of t(2;5)(p23;q35) and overexpresses anaplastic lymphoma kinase (ALK). MUC-1 is known to be expressed in normal and malignant epithelial cells and has been associated with a poorer patient survival in various human malignancies. For this study, eligible patients with ALCL were HIV negative, received anthracycline-containing regimens, and had pretreatment archival tissue. Expression of MUC-1 and ALK was determined immunohistochemically after heat-induced antigen retrieval. A 10% cutoff for MUC-1 positivity was used. We identified 63 patients with systemic ALCL (22 ALK+, 41 ALK−) with a median age of 47 years, and 41 were male. MUC-1 was detected in 16 of 22 (73%) ALK-positive and 20 of 41 (49%) ALK-negative ALCL (P = 0.06, χ² test). MUC-1 expression was not associated with apoptotic rate as detected by terminal deoxynucleotidyl transferase-mediated nick end labeling assay or proliferation index as evaluated by MIB-1 antibody. For 48 patients with ALCL (16 ALK+, 32 ALK−) and complete clinical follow-up, 5-year progression-free survival (PFS) was 39.7% for patients with MUC-1-positive tumors versus 75.2% (P = 0.027 by Log-rank) for patients with MUC-1-negative tumors. For the ALK-negative ALCL group of 32 patients, the 5-year PFS was 26 versus 70.8% for patients with MUC-1-positive versus MUC-1-negative tumors (P = 0.0096 by Log-rank). For the ALK-positive ALCL group of 16 patients, the 5-year PFS was 52 versus 100% for patients with MUC-1-positive versus MUC-1-negative tumors (P, not significant). In summary, MUC-1 is frequently expressed in systemic ALCL, and its expression is associated with significantly inferior outcome in patients untreated previously with ALK-negative tumors. Future studies should explore the underlying molecular mechanisms of MUC-1 expression in these tumors and its role as a target for novel therapeutic strategies.

INTRODUCTION

Primary systemic ALCL is a relatively uncommon lymphoma in adults, but it accounts for 30–40% of large cell lymphomas of childhood (1). Systemic ALCL is frequently associated with the t(2;5)(p23;q35) and variant chromosomal translocations involving the 2p23 locus, resulting in overexpression of ALK (reviewed in Ref. 2). The t(2;5) disrupts the NPM gene at 5q35 and ALK gene at 2p23, generating a novel NPM-ALK gene consisting of the NH2-terminal portion of NPM fused to the cytoplasmic catalytic domain of ALK, which retains tyrosine kinase activity (3, 4). ALK expression, the t(2;5) translocation, or both have been detected in a variable proportion of ALCL, ranging from 12 to 80% in different series (5–11). Several clinical prognostic factors have been identified for patients with systemic ALCL, including performance status, international prognostic index, extranodal involvement, and biological markers (12), e.g., patients with ALK-positive ALCL have been reported to have a better clinical outcome than patients with ALK-negative ALCL (12–14). However, additional markers related to the immunophenotype and biology of ALCL merit further investigation to identify new prognostic factors that could lead to better patient stratification and rational treatment design.

MUC-1, also known as epithelial membrane antigen, was first identified as a large molecular weight transmembrane glycoprotein of human milk (15). The extracellular domain of the MUC-1 protein contains a variable number (20–120) of amino acid tandem repeats, making MUC-1 highly polymorphic (16). MUC-1 also contains a transmembrane domain and a 72 amino acid intracytoplasmic tail. The extracellular domains of MUC-1 may play a role in cell adhesion and migration mechanisms (17, 18). The precise function of the intracellular domains of MUC-1 needs further investigation to understand the underlying molecular mechanisms of MUC-1 expression in systemic ALCL and its role as a target for novel therapeutic strategies.
is not clear, but these domains may play a role in signal transduction (19).

Although MUC-1 has been detected in normal glandular epithelial cells (20), its expression and glycosylation status have been reported to be altered in various human cancers (21, 22). Overexpression of MUC-1 may cause significant changes in tumor cell properties, such as reduced aggregation of epithelial tumor cells or inhibition of their interaction with the extracellular matrix (18), as a consequence of negatively charged MUC-1 molecules causing repulsive effects between cells. These changes might contribute to the mechanisms involved in tumor progression (22). Although MUC-1 knockout mice develop normally, induction of breast carcinomas in these mice results in slowly growing tumors with lower metastatic potential (22). Although MUC-1 knockout mice developed normally, induction of breast carcinomas in these mice results in slowly growing tumors with lower metastatic potential (22).amage of patients with ALK-positive and -negative anaplastic large cell lymphoma

<table>
<thead>
<tr>
<th></th>
<th>ALK positive</th>
<th>ALK negative</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median years)</td>
<td>34</td>
<td>50</td>
<td>0.0042</td>
</tr>
<tr>
<td>Range (years)</td>
<td>21</td>
<td>69–100</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male 13</td>
<td>28</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>Female 9</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ann Arbor stage</td>
<td>I–II 30.8%</td>
<td>41.9%</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>III–IV 69.2%</td>
<td>58.1%</td>
<td></td>
</tr>
<tr>
<td>B symptoms</td>
<td>53.8%</td>
<td>34.5%</td>
<td>0.31</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>21.4%</td>
<td>30%</td>
<td>0.72</td>
</tr>
<tr>
<td>Serum LDH &gt; 1.5× normal</td>
<td>66.7%</td>
<td>43.3%</td>
<td>0.2</td>
</tr>
<tr>
<td>Serum β2-microglobulin &gt; 2.5 mg/liter</td>
<td>71.4%</td>
<td>59.3%</td>
<td>0.51</td>
</tr>
<tr>
<td>Albumin &lt; 3.5 grams/dl</td>
<td>18.2%</td>
<td>26.1%</td>
<td>0.9</td>
</tr>
<tr>
<td>WBC (mean ± SD)</td>
<td>13.8 ± 17.2</td>
<td>10.6 ± 4.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Anemia</td>
<td>30%</td>
<td>58.3%</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* The P values were calculated with Fisher’s exact test for all comparisons, except for age, which was calculated using the Mann-Whitney test.

Transduction (19).

The histopathologic diagnosis of ALCL was based on both morphological and immunohistochemical criteria as stated in the WHO classification (1). In particular, ALK-negative ALCL cases closely resembled classic or monomorphic variants of ALK-positive ALCL morphologically, and the neoplastic cells were strongly and uniformly positive for CD30.

All cases were routinely processed, fixed in 10% buffered formalin, and embedded in paraffin. Immunohistochemically, all ALCL cases expressed CD30 and were negative for B-cell antigens (CD20 and/or CD79a). All T-cell tumors were positive for one or more T-cell antigens (CD3, CD5, CD43, or CD45R0). Tumors negative for CD3 and CD5 but positive for untreated previously and correlated these findings with clinical and laboratory features and outcome. Our results suggest a significant and independent association of MUC-1 expression with worse prognosis in ALCL patients, particularly in patients with ALK-negative tumors.

**PATIENTS AND METHODS**

*Study Group.* This group included 63 cases of primary systemic ALCL accessioned at The University of Texas M. D. Anderson Cancer Center between 1984 and 2000. All patients received doxorubicin-based chemotherapy. All tumor specimens analyzed were obtained before treatment. The clinicopathological features of these patients are shown in Table 1. The median age of patients with ALK+ tumors was 32 years compared with 50 years for patients with ALK− tumors (P = 0.0042). All patients were adults except three of the ALK+ ALCL patients who were <16 years of age. All other clinical parameters, including Ann Arbor stage, were comparable. Nine of 12 patients with skin involvement as a secondary manifestation of systemic ALCL had stage IV disease (P = 0.004, Fisher’s exact test).

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CD43 or CD45RO were considered to be of T-cell lineage in this study. Null cases were negative for all T-cell antigens.

**Design and Construction of the Tissue Array.** Full tissue sections from 20 ALCL and a tissue array that included triplicate tumor cores from 43 systemic ALCL and two reactive lymph nodes were assessed. A manual tissue arrayer (Beecher Instruments, Silver Spring, MD) was used to construct the tissue array as described previously (35). Full tissue sections and tissue array sections, cut with a standard microtome, were 5 μm thick.

**Immunohistochemical Methods.** Our immunohistochemical methods have been described previously (36). The following panel of monoclonal antibodies were used: (a) ALK-1 (1:30; DAKO, Carpinteria, CA); (b) MUC-1 (1:50, clone NCL-HMFG-2; Novocastra, Newcastle upon Tyne, United Kingdom); and (c) MIB-1 (1:120; Immunotech, Westbrook, ME). Heat-induced epitope retrieval was performed for all antibodies as described elsewhere (36). The slides were incubated with monoclonal antibody at room temperature for 60 min. Detection of the immunoreaction was performed using the LSAB+ kit (DAKO), which contains the secondary biotinylated antibody (incubation time: 20 min.) and the streptavidin/horseradish peroxidase complex (incubation time: 20 min.). We used DAB/H2O2 (DAKO) as the chromogen and hematoxylin as the counterstain. Intestinal mucosa was used as an external positive control for MUC-1 immunostaining. In addition, a variable number of plasma cells present in all tissue sections served as internal positive controls. Slides stained only with normal rabbit serum (DAKO) without primary antibody were used as negative controls to exclude nonspecific cross-reaction.

Any cytoplasmic MUC-1 staining of ALCL cells was considered positive, irrespective of intensity. On the basis of the distribution of MUC-1 expression in ALCL tumors and for the purpose of statistical analysis, a 10% cutoff was used to define MUC-1 positivity. PI was designated as the percentage of MIB1-positive nuclei determined by counting 500-2000 tumor cells in each case.

**Tdt-mediated dUTP Nick End Labeling Assay.** AR was evaluated using a Tdt-mediated dUTP nick end labeling assay as described previously (36). Briefly, tissue sections were deparaffinized in a graded series of ethanol, rehydrated, and pretreated with proteinase K (20 μg/ml) for 25 min at 37°C. Slides were then incubated for 5 min in 3% H2O2 in PBS at pH 7.4 to block endogenous peroxidase activity. Tdt (New England Biolabs, Beverly, MA) was subsequently applied (15 μl/slide) for 1 h, at 37°C, in 20 mm Tris-acetate (pH 7.9), 50 mm potassium acetate, 10 mm magnesium acetate, 1 mm DTT, 0.25 mm CoCl2, and 24 μm biotin-dATP (Life Technologies, Inc., Gaithersburg, MD). For the detection of labeled termini, streptavidin-biotin-horseradish peroxidase complex (LSAB+ kit) and DAB were used, and the slides were counterstained with hematoxylin. As a positive control, we used sections from a Karps 299 cell block incubated with DNase I (2.5 μg in 50 μl/slide Tris-buffered saline containing 6 mm MgCl2) for 30 min at 37°C. Sections from the same cell line block, incubated with a reaction mixture lacking TdT, served as negative controls in each experiment. The percentage of positively stained nuclei was designated as the AR.

**Statistical Analysis.** The χ2 and Fisher’s exact tests were used to compare MUC-1 expression as a categorical variable (positive versus negative) with various clinicopathological parameters. The Mann-Whitney U test was chosen for the non-parametric correlation of AR and PI between MUC-1-positive and -negative ALCL. PFS, defined as time from initiation of therapy to last follow-up, primary treatment failure, or relapse was chosen to evaluate the clinical outcome of the patients, because various postrelapse factors might impact OS of the patients. Survival analysis was based on the method of Kaplan and Meier. The statistical independence between variables was evaluated by multivariate analysis using Cox proportional hazards model. All computations were carried out using StatView statistical program (Abacus Concepts, Inc., Berkeley, CA).

**RESULTS**

**MUC-1 Expression in Systemic ALCL.** Using a 10% cutoff, 36 (57.1%) of 63 ALCL tumors were positive for MUC-1 (Fig. 1). The intensity of staining was variable, ranging from weak to strong. MUC-1 was strongly positive in coexisting normal plasma cells, present in many cases. MUC-1 immunoreactivity was restricted to the membrane and cytoplasm with variable intensity. MUC-1 expression was positive in 16 of 22 (73%) ALK-positive and in 20 of 41 (49%) ALK-negative tumors (P = 0.06, χ2 test). MUC-1 expression did not correlate with clinical and laboratory features of these patients at time of diagnosis (Table 3).

**AR and PI.** AR was assessed in 48 ALCL tumors. The mean percentage of apoptotic neoplastic cells was 1.89 ± 1.8% for the entire study group, ranging from 0.1 to 7.9% (median 1.15%). Staining was restricted to the nucleus of apoptotic cells. AR correlated with ALK expression, as reported previously (36), but was not statistically associated with MUC-1 positivity (Table 3). PI, evaluated in 37 cases of ALCL, ranged from 20.4 to 94.6% with a mean 69.2 ± 16.9% (median 73%). No correlation between PI and MUC-1 was observed (Table 2).

**Survival Analysis.** Complete clinical follow-up (median 47 months) was available for 48 ALCL patients (16 ALK+, 32 ALK−). For the entire group, 5-year PFS was 39.7% for patients with MUC-1-positive tumors and 75.2% for patients with MUC-1-negative tumors (P = 0.027 by Log-rank; Fig. 2a). For the ALK-negative ALCL group, the 5-year PFS was 26% for patients with MUC-1-positive tumors compared with 70.8% for patients with MUC-1-negative tumors (P = 0.0096 by Log-rank; Fig. 2b). For the ALK-positive ALCL group, the 5-year PFS was 52% for patients with MUC-1-positive tumors compared with 100% for patients with MUC-1-negative tumors (P not significant, Log-rank; Fig. 2c).

OS at 5 years for the entire group was 70.7% for patients with MUC-1-positive tumors and 93.8% for patients with MUC-1-negative tumors (P = 0.01 by Log-rank; Fig. 3a). For the ALK-negative ALCL group, OS at 5 years was 55.6% for patients with MUC-1-positive tumors versus 93.3% for patients with MUC-1-negative tumors (P = 0.0056 by Log-rank; Fig. 3b). For the ALK-positive ALCL group, the 5-year OS was 88.9% for patients with MUC-1-positive tumors compared with 100% for patients with MUC-1-negative tumors (P not significant, Log-rank; Fig. 3c). Similar results for PFS and OS were obtained when the three pediatric cases were excluded from the
survival analysis (data not shown). Univariate analysis also confirmed the prognostic significance of many known clinical and laboratory factors, such as high serum β2-microglobulin (P = 0.05, Log-rank), low serum albumin (P = 0.0091, Log-rank), anemia (P = 0.0011, Log-rank), and bone marrow involvement (P = 0.0004, Log-rank).

Using Cox proportional hazards model, which included all known prognostic clinical and laboratory parameters for non-Hodgkin’s lymphomas, forward and backward stepwise regression analysis revealed the independent prognostic value of MUC-1 expression, along with ALK expression, age, serum albumin, and B-symptoms, as summarized in Table 4.

### DISCUSSION

We investigated the clinical significance of MUC-1 expression in a series of ALCL tumors untreated previously. Using a 10% cutoff, we found that ALK-positive ALCLs more frequently express MUC-1 (73%) than ALK-negative ALCLs (49%), which approached statistical significance (P = 0.06). These results are in agreement with those of previous studies that have reported overexpression of MUC-1 in most ALK-positive ALCLs using various monoclonal antibodies specific for MUC-1 epitopes (29, 33).

The mechanisms of MUC1 expression in ALCL are unknown. It is reasonable to speculate that alterations of the 1q21 locus may result in overexpression of MUC1, and Teruya-Feldstein et al. (28) have shown previously that 1q21 abnormalities are frequently detected in B-cell non-Hodgkin’s lymphomas, including a series of diffuse large B-cell lymphoma. However, the relatively low frequency of 1q21 locus alterations can only partly explain MUC1 overexpression in these tumors.

![Image](image_url)

**Table 3** Association between MUC-1 expression and kinetic parameters of anaplastic large cell lymphoma

<table>
<thead>
<tr>
<th>MUC-1 expression</th>
<th>Apoptotic rate (n = 32)</th>
<th>PI (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC-1 positive</td>
<td>1.8 ± 1.7</td>
<td>72.4 ± 12.9</td>
</tr>
<tr>
<td>MUC-1 negative</td>
<td>1.6 ± 1.5</td>
<td>66.6 ± 20.7</td>
</tr>
<tr>
<td>P</td>
<td>0.38</td>
<td>0.60</td>
</tr>
</tbody>
</table>

* Mann-Whitney test.
Therefore, other mechanisms are probably involved, as suggested by data from several studies on epithelial tumors, e.g., it has been reported that MUC1 may be up-regulated in breast cancer cells by the c-ErbB2 and ras signaling pathways (37). In addition, it has been shown that the MUC1 gene promoter contains a STAT-responsive element for both STAT-1 and STAT-3 proteins in carcinoma cells (38). Because STAT-3 is frequently overexpressed and phosphorylated in ALCL (39), it is tempting to speculate that elevated STAT-3 levels may contribute to overexpression of MUC-1 in systemic ALCL. However, the aforementioned mechanisms need to be further investigated using in vitro ALCL systems.

We also report that MUC-1 expression is significantly associated with an inferior PFS and OS in 48 ALCL patients with complete clinical follow-up (Figs. 2a and 3a). This association becomes even more significant when the survival analysis is restricted to patients with ALK-negative ALCL (Figs. 2b and 3b). In addition, none of the ALK-positive ALCL patients who had MUC-1-negative tumors failed therapy (Figs. 2c and 3c). To the best of our knowledge, this is the first study to show that MUC-1 is an adverse prognostic factor in systemic ALCL. Multivariate analysis confirmed the independent prognostic value of MUC-1 expression in ALCL, along with other known prognostic factors of these tumors (Table 4). Notably, statistical analysis did not reveal any significant association between MUC-1 expression and presenting clinical and laboratory parameters of the patients. Furthermore, MUC-1 positivity did not correlate with AR or tumor cell proliferation, suggesting that the
adverse prognostic impact of MUC-1 in ALCL is probably not related to tumor kinetics.

Others have suggested that ALK-negative ALCL, as defined in the WHO classification, is better classified as peripheral T-cell lymphoma, because the biology of these tumors is clearly different from ALK-positive ALCL (40). Thus, the prognostic significance of MUC-1 expression in ALK-negative ALCL suggests that MUC-1 expression may also be of prognostic significance in other types of peripheral T-cell lymphomas. We have not addressed this issue, because other types of peripheral T-cell lymphoma were not the focus of our study.

On theoretical grounds, several reasons may explain why MUC-1 expression confers a poorer prognosis in ALCL, presumably because of chemotherapy resistance. First, high serum levels of MUC-1 protein may act as a negative regulator of tumor kinetics or decreasing their permeability into the tumor cell.

The possible involvement of MUC-1 overexpression in the pathogenesis of ALCL is unknown. However, it has been suggested that MUC-1 cytoplasmic domains are involved in signal transduction pathways, which are frequently altered in human cancers. More specifically, the cytoplasmic domain of the MUC-1 molecule binds directly to β-catenin (43) and competes with E-cadherin for the same β-catenin-binding site (44). MUC-1 can be phosphorylated by glycogen synthase kinase-3β, resulting in decreased binding of MUC-1 with β-catenin (44). In contrast, c-Src kinase and protein kinase-C6, both capable of binding and phosphorylating MUC-1, stimulate binding of MUC-1 to β-catenin (45, 46). Recent studies have also demonstrated that transgenic MUC-1 interacts with epidermal growth factor receptor signaling through the activation of mitogen-activated protein kinase pathways (47). Ablerrant expression of MUC-1 by cancer cells has been shown to be immunogenic, making MUC-1 a good candidate target for immunotherapy. Because cancer patients have a low number of CTLs and low titer of IgM antibodies against MUC-1, several vaccines have been developed to boost MUC-1-specific immunity and reported to be active in several studies. These vaccines include MUC-1 peptides admixed with adjuvants or MUC-1 peptide-pulsed dendritic cells (48), MUC-1 cDNA (49), recombinant vaccinia viruses expressing a modified MUC-1 (50), or fusions of dendritic and carcinoma cells (51). Vaccinations targeting MUC-1 have been used in preliminary Phase I clinical trials with promising results (52, 53). Recently, the use of clonal CTLs directed against MUC-1 epitopes was tolerated well and showed good response in preclinical murine tumor models (54). In addition, radioimmunotherapy using 90Y-linked monoclonal MUC-1 antibodies may increase the complete response rate and cure minimal metastatic disease in cancer patients as reviewed by Richman and DeNardo (55).

We conclude that MUC-1 is frequently expressed in primary systemic ALCL and its expression is associated with a poorer prognosis. Future studies are needed to explore the underlying mechanisms of MUC-1 overexpression in ALCL. Modulation of MUC-1-specific immunity may provide novel immunotherapeutic strategies for the treatment of ALCL.

REFERENCES

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