

Aberrant overexpression of MUC1 contributes to tumour progression in adult T-cell Leukaemia/Lymphoma cells.

Hiroo Hasegawa,^{1*} Minoru Komoda,^{2*} Yasuaki Yamada,¹ Suguru Yonezawa,³
Hideaki Tsutsumida,³ Kazuhiro Nagai,⁴ Sunao Atogami,¹ Kazuto Tsuruda,¹
Akemi Osaka,¹ Daisuke Sasaki,¹ Katsunori Yanagihara,¹ Yoshitaka Imaizumi,²
Kunihiro Tsukasaki,² Yasushi Miyazaki,² Shimeru Kamihira¹

¹Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

²Department of Hematology, Atomic Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

³Department of Human Pathology, Field of Oncology, Course of Advanced Therapeutics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan.

⁴Transfusion Service, Nagasaki University Hospital of Medicine and Dentistry, Nagasaki, Japan

* These authors contributed equally to this work.

Corresponding author: Yasuaki Yamada, M.D., Ph.D.

Department of Laboratory Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki City, 852-8501, Japan

Tel.: +81-95-849-7408; Fax: +81-95-849-7422

E-mail: y-yamada@nagasaki-u.ac.jp

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Abstract

Aberrant overexpression of membrane-associated mucin MUC1 is implicated in the pathogenesis of cancer, particularly of adenocarcinomas. Adult T-cell Leukaemia/Lymphoma (ATL), an aggressive neoplasm etiologically associated with human T-lymphotropic virus type-1 (HTLV-1), exhibits invasive tropism into various organs, resulting in disease progression and resistance to treatment.

In the present study, we showed that MUC1 is overexpressed exclusively in cells of ATL among haematological malignancies. Furthermore, increased expression of MUC1 correlated with a poor prognosis, suggesting MUC1 to be a prognostic marker in ATL. Various functional analyses with knockdown experiments using a specific siRNA for MUC1 revealed that MUC1 is involved in cell growth, cell aggregation, and resistance to apoptosis. Although it has been shown that the anti-adhesive properties of MUC1 facilitate migration and metastasis of tumour cells, our findings indicated that MUC1 contributes to cell-cell adhesion. Mucins thus seem to play a role in the pathogenesis and/or progression of ATL.

Introduction

Mucins are high molecular weight glycoproteins expressed at the apical borders of secretory epithelial cells [1]. The membrane-associated mucin MUC1 (also known as RP11-263K19.2, CD227, EMA, H23AG, KL-6, MAM6, PEM, PEMT, or PUM) represents a type I transmembrane glycoprotein and consists of 3 domains; a large extracellular domain containing a tandem repeat domain, transmembrane domains that are highly conserved among species, and a short cytoplasmic tail (CT) that was recently found to correlate with signal transduction [2; 3]. The biological functions of MUC1 are the lubrication and hydration of cell surfaces as well as protection from microorganisms and degradative enzymes [1]. In addition, MUC1 is implicated in the pathogenesis of cancer, particularly of adenocarcinomas [4; 5], and increased concentrations of mucin-type glycoproteins in serum are basically correlated to increasing tumour burden and a poor prognosis [6; 7]. Although the precise mechanism regulating the adhesive or anti-adhesive effect of MUC1 is unknown, the anti-adhesive effect may be exerted by blocking cell-cell and cell-extracellular matrix interactions [8]. This property of MUC1 confers migrational and metastatic capabilities to tumour cells [9].

The detection of the epithelial membrane antigen (EMA) in plasma cells and some lymphomas was first described in the early 1980s [10; 11]. Accumulated evidence indicates that myeloma cells express MUC1, making MUC1 a potential molecular target in the treatment of myeloma [12; 13; 14]. However, the implications of MUC1 expression for disease

progression and prognosis as well as therapeutic intervention are not clear particularly regarding haematological malignancies.

Adult T-cell Leukaemia/Lymphoma (ATL), an aggressive neoplasm etiologically associated with human T-lymphotropic virus type-1 (HTLV-1), is resistant to treatment [15; 16]. ATL, with 4 clinical subtypes; acute, chronic, smoldering and lymphoma, is known to have diversity in clinico-pathological features [17]. Until recently, ATL was thought to derive from a subset of helper/inducer T-cells, but evidence is now accumulating that ATL cells originate from regulatory T-cells (Treg cells) naturally expressing CD4, CD25, CCR4, CCR8, CD30, GITR, and Foxp3 [18; 19; 20]. This well explains the characteristic clinical features of ATL, such as severe immunodeficiency and invasive tropism of tumour cells into skin, given the properties of Treg cells . However, ATL cells also tend to invade lung, liver, spleen, brain, and bone and grow co-adhesively [21; 22]. Studies of the cell-cell adhesive interaction of ATL cells have been focusing on adhesion molecules such as selectin, integrin, and the immunoglobulin super-family, including E-selectin, L-selectin, LFA-1, ICAM-1, CD151, and TSLC1 [23; 24; 25]. However, little attention has been paid to whether mucins participate in such intercellular interaction.

In the present study, we found that MUC1 is overexpressed at the levels of mRNA and protein in ATL cells and involved in cell-growth, cell aggregation, and resistance to apoptosis. This represents the first report of the expression and functions of MUC1 in ATL cells.

Materials and methods

Patients and samples

A total of 64 patients with ATL, 52 with acute-type ATL and 12 with chronic-type ATL, and 10 healthy donors were included in this study. The diagnosis of ATL was based on clinical features, haematological findings including cytologically or histologically proven mature T-cell leukaemia/lymphoma, and serum anti-HTLV-1 antibodies, and monoclonal HTLV-1 provirus integration into the genome was confirmed by Southern blot hybridization in all cases [26]. The subclassification of ATL was made according to previously described criteria [17]. Peripheral blood mononuclear cells (PBMCs) from patients with ATL and healthy donors were purified by density gradient centrifugation using Lympho-prep (AXIS-SHIELD, Oslo, Norway). Each sample contained more than 90% leukaemia cells at the time of analysis. The study was approved by the Ethics Committee of Nagasaki University Hospital, and all materials from patients were obtained with informed consent.

Cell lines

The ATL-derived cell lines SO4, ST1, KK1, KOB, OMT, and LM-Y1 were established in our laboratory from sample of ATL patients [26; 27; 28; 29]. They were maintained in RPMI 1640 medium supplemented with 10% FBS and 0.5U/ml of IL-2 (kindly provided by Takeda Pharmaceutical Company, Ltd., Osaka, Japan). We also used the HTLV-1-infected T-cell

lines MT2 and HuT102, human T cell leukaemia cell lines Jurkat and MOLT-4, human B lymphoblastoid cell line SKW6.4, Burkitt lymphoma cell lines Ramos, CA46, and HS-Sultan, transformed follicular lymphoma cell line SUDHL-4, acute myeloid leukaemia cell line HL-60, and monocytic leukaemia cell lines THP-1 and U937. These cell lines were obtained from American Type Culture Collection (ATCC) and maintained in RPMI 1640 medium supplemented with 10% FBS.

Chemicals, cell proliferation assay, and homotypic adhesion assay

Chemicals used in this study were MG132 and soluble TRAIL (Biomol Research Laboratories, Plymouth Meeting, PA, USA), and Nutlin-3a (Alexis Biochemicals, San Diego, CA, USA). The cell proliferation assay (MTS assay) was performed with a Cell Titer 96® AQueos Cell proliferation Assay kit (Promega, Madison, WI, USA) according to the manufacturer's directions. We used KOB and KK1 cells for homotypic adhesion assays. These cells usually form clumps while proliferating and show a decrease in the number of non-aggregated cells. After being transfected with MUC1 or control siRNA, the cells (5×10^5 /mL) were seeded and incubated. After 24, 48, and 72 hours, 5 representative fields of the cultures were photographed microscopically. By using these photographs, we counted the number of clumps and every cell not part of an aggregate. An aggregate consisting of more than 3 cells was counted as a clump. The number of clumps and non-aggregated cells were added to obtain the total number of cells and the percentage of non-aggregated

cells was determined. All measurements represented the average values obtained from 5 representative fields.

Real-time quantitative RT-PCR

Total RNA from clinical samples, healthy donors, and cell lines was isolated using ISOGEN (Wako, Osaka, Japan). After contaminating DNA was removed, cDNA was constructed using the Thermoscript RT-PCR System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Real-time quantitative PCRs for MUC1 and GAPDH were performed on a Roche LC480 (Roche Diagnostics, Basel, Switzerland) according to the directions [30; 31]. The set of primers and TaqMan probe for MUC1 were as follows: sense: 5'-GTGCCCCCTAGCAGTACCG-3', antisense: 5'-GACGTGCCCCCTACAAGTTGG-3', and TaqMan probe: 6FAM-AGCCCCTATGAGAAGGTTTCTGCAGGTAATG-TAMRA [32]. All data were normalized to the level of GAPDH measured in the same sample.

Flow cytometric analysis

The cell surface expression of MUC1 was examined by flow cytometry (FCM) using anti-MUC1/CD227 (BD Biosciences, San Diego, CA, USA), anti-MUC1/DF3 (TFB, Tokyo, Japan), anti-MUC1/Core (Clone Ma552, Novocastra Laboratories, New Castle, UK), or anti-MUC1/HMFG-1 (kindly provided by Dr. J. Taylor-Papadimitriou, Imperial Cancer Research Fund Laboratories, London, UK) [33]. Mouse IgG1 (DAKO Japan,

Kyoto, Japan) and FITC-conjugated goat anti-mouse IgG1 (DAKO Japan) were used as a negative control and as a secondary antibody, respectively. PBMCs from ATL patients and healthy donors were stained further with anti-CD25 PE (BD), anti-CD19 PE (BD) and/or anti-CD4 perCP (BD). CD4⁺ CD25⁺ cells in ATL cases and CD4⁺ CD19⁻ cells among normal PBMCs were analyzed. To evaluate apoptotic changes, we used an Annexin-V and PI Kit (Bender Medsystems, Vienna, Austria). All experiments were performed using a FACSCalibur flowcytometer and Cellquest software (BD).

Immunocytochemistry

Cells smeared on glass slides were fixed with 4% paraformaldehyde at 4°C for 15 min and then stained with anti-MUC1/CD227 antibody for 2 hours at room temperature after permeabilization using a commercial kit (BD). The slides were counter-stained with Hoechst 33258 for staining of the nucleus (Molecular Probe Inc., Eugene, OR, USA). Signals were fluoroscopically observed using Leica DM4000B Microsystems (Mannheim, Germany) and the fluorescence images were collected and analyzed with a Leica AF6000 Application Suite.

Small interfering RNA experiments

Transfection was performed with a Cell line Nucleofector kit V and the Nucleofector™ system (Lonza, Cologne, Germany). The transfection programs for KOB (T-16) and KK1 (T-20) were determined so that the

viability of cells and the transfection efficiency would be compatible. Twenty four hours after transfection, the cells were processed for FCM, a cell proliferation assay, a homotypic adhesion assay, or an evaluation for apoptosis. We prepared three different small interfering RNA (siRNA) against MUC1; Silencer Select pre-designed siRNA s9065, s9066, and s9067 and control siRNA (Silencer negative control #1) (Applied Biosystems, Foster City, CA, USA). After evaluating the effect of each siRNA by monitoring the target's mRNA and protein, we eventually selected siRNA s9065.

Statistical analysis

Survival was analyzed using the Kaplan-Meier method, with the log rank test used to calculate the significance of differences. Other data were statistically analyzed based on Student's t-test, and differences were recognized as significant if the p value was less than 0.05 or 0.01. The results were expressed as the mean \pm standard deviation (SD).

Results

ATL cell lines have high levels of MUC1

We first analyzed expression profiles of MUC1 mRNA by quantitative RT-PCR in various haematological cell lines: 8 ATL-related, 2 T-cell leukaemia, 5 B-cell lineage, and 3 myeloid lineage leukaemia cell lines. The ATL-related cell lines had quite high levels of MUC1 mRNA. The

other cell lines had quite low levels (Figure 1A). Flowcytometric analysis confirmed that all ATL-related cell lines had high levels of MUC1 at the cell surface (Figure 1B). In contrast, Jurkat, MOLT4 and HS-Sultan cells weakly expressed and HL60 cells expressed almost no, MUC1 (Figure 1C). Supporting the results of FCM, microscopic analysis showed that MUC1/CD227 was uniformly distributed in the cytoplasm of ATL cells but not in the other cell lines (Figure 1D and not shown). Different patterns of MUC1 expression are sometimes observed due to different monoclonal antibodies used [34]. The antigen MUC1/CD227 used in the present study is known to recognize the core protein of MUC1 [35]. Then we investigated the expression profiles of other antigens, MUC1/DF3, MUC1/Core, and MUC1/HMFG-1, in KK1, LM-Y1, HuT102, and HL60 cells by FCM. As shown, all but HL60 cells were stained by these antibodies (Figure 1E). Consequently, ATL cell lines have high levels of MUC1 mRNA and protein.

Primary ATL cells express MUC1 and high levels of MUC1 mRNA correlate with a poor prognosis

High levels of MUC1 expression correlate with a poor prognosis in most epithelial cancers; breast cancer [36], esophageal cancer [37], gastric cancer [33], colorectal cancer [38], lung cancer [39; 40], and so on. However, no report had investigated MUC1 expression and disease progression or prognosis in haematological malignancies. We examined MUC1 expression in primary ATL cells and evaluated the prognosis in ATL

cases. Irrespective of subtype, ATL cells had significantly high levels of MUC1 mRNA compared to PBMCs from healthy donors (Figure 2A). Among 64 ATL cases, 30 each with high and low levels of MUC1 mRNA were chosen. Then we compared survival periods between the two groups. The group with high levels of MUC1 mRNA had significantly shorter survival times (Figure 2B). Unexpectedly however, there was no significant difference between acute type cells and chronic type cells in MUC1 mRNA expression (Figure 2A). These results suggest the expression of MUC1 mRNA to be a prognostic marker for ATL.

Representative data on the cell surface protein expression of MUC1 in normal CD4-positive lymphocytes, chronic ATL cells, and acute type ATL cells are shown in Figure 2C. Interestingly, acute type cells showed significantly higher levels of MUC1 protein than chronic type cells, and there was no difference between chronic type cells and normal PBMCs (Figure 2D).

MUC1 contributes to the cell proliferation in ATL cell lines

We performed knockdown experiments using siRNA for MUC1 (si-MUC1) to investigate the role of MUC1 in cell proliferation. Effects of si-MUC1 on mRNA levels were well documented within 24 hours and continued after 72 hours (Figure 3A). As shown, the cell surface expression of MUC1 was also suppressed by si-MUC1 in KOB and KK1 cells (Figure 3B). In this setting, we performed cell proliferation assays up to the 96 hour mark, comparing si-MUC1 cells and si-control cells.

The inhibition of cell growth in si-MUC1 cells at 96 hours was approximately 25% and 30% of that in KOB and KK1 cells, respectively (Figure 3C). These results suggest that MUC1 contributes to cell growth in ATL cell lines.

A unique feature of MUC1 contributing to cell aggregation in ATL cell lines

It has been demonstrated that anti-adhesive properties of MUC1 inhibit cell-cell contact and facilitate the migration and metastasis of tumour cells [41]. Then we investigated the effects of MUC1 on the aggregation of KOB and KK1 cells by using si-MUC1. KOB and KK1 cells usually form “clumps” when they proliferate. Microscopic examination showed that the aggregation was inhibited by si-MUC1 (Figure 4A). Since it is difficult to count aggregated cells when clumps form, we determined in percentage terms, the number of non-aggregated cells. In the si-control setting, clumps formed during the cell proliferation, which resulted in a reduction in the percentage of non-aggregated cells. After 72 hours, the percentage of non-aggregated cells among si-control cells was 41% and 14% that among KOB and KK1 cells, respectively (open bars in Figure 4B). In contrast, si-MUC1 cells caused less aggregation, resulting in an increase in non-aggregated cells to 80% and 64% of that for KOB and KK1 cells, respectively (closed bars in Figure 4B). These results suggest that MUC1 contributes to the aggregation of these cells.

MUC1 inhibits apoptosis in ATL cells

A recent report indicated that MUC1 attenuates apoptotic responses such as TRAIL-induced apoptosis in some types of cancer cells [42]. Previously, we showed that ATL cells are resistant to TRAIL though they have TRAIL death-receptors [26]. We then investigated whether the MUC1 of ATL cells influences the resistance to TRAIL-induced apoptosis. Si-control and si-MUC1 cells of the KOB and KK1 lines were treated with 400ng/mL of TRAIL, and Annexin-V/PI staining was performed. Si-MUC1 cells increased the proportion of Annexin-V-positive cells in the KOB and KK1 lines from 32% to 45% and from 20% to 42%, respectively (Figure 5A). We performed similar experiments using MG132, a more potent inducer of apoptosis than TRAIL for these cells. Si-MUC1 cells also increased the percentage of Annexin-V-positive cells in KOB and KK1 (Figure 5B). These results indicate that overexpression of MUC1 contributes to the inhibition of apoptosis in ATL cells. Meanwhile, we could not find any differences between si-control and si-MUC1 cells when the MDM2 inhibitor Nutlin-3a was used. KOB cells with wild-type p53 did not show any significant difference in Annexin-V-positive cells between si-control and si-MUC1 cells. KK1 cells with a mutant p53 did not respond to Nutlin-3a (Figure 5C).

Discussion

In the present study, we revealed that MUC1 is overexpressed exclusively in ATL cells. The aberrant overexpression is implicated in the

pathogenesis of ATL and makes MUC1 a potential molecular target in the treatment of ATL.

MUC1 expression had been thought to be restricted to epithelial cells, however, non-epithelial expression of MUC1 has recently been described in haematological malignancies such as myelomas [43], B-cell lymphomas [44], anaplastic large cell lymphomas [45], and acute myelogenous leukaemia (AML) [46]. Treon *et al.* investigated MUC1 expression by using various haematological cell lines other than ATL cells [43]. Consistent with their results, T-cell lines, B-cell lines, and AML cell lines expressed little or no MUC1 in the present study. Accumulated evidence indicates that myeloma cells express MUC1, making MUC1 a potential molecular target in the treatment of myeloma [12; 13; 14]. Meanwhile, as a marker for the micro-metastasis of malignant epithelial cells, MUC1 expression has been studied in normal peripheral blood and hematopoietic tissues [47; 48; 49]. In regard to peripheral blood cells, one previous report showed the constitutive expression of MUC1 mRNA and an absence of MUC1 protein at the cell surface in resting human T cells [35]. Another study found that MUC1 was undetectable by RT-PCR or Northern blotting in resting T cells and the level of MUC1 expression in activated human T cells was low [50]. Our results indicated that normal PBMCs have quite low levels of MUC1 mRNA and that normal CD4 lymphocytes have only marginal amounts of MUC1 at the cell surface. These discrepancies remain to be explained and the profiles of MUC1 expression in normal peripheral blood seem to be controversial.

We previously investigated the serum levels of Krebs von den Lungen-6 (KL-6) in patients with haematological malignancies and found them to be high in some cases without pulmonary complications, most of which were ATL cases, and closely related to the stage of ATL [51]. In the present study, most chronic type ATL cells had upregulated mRNA expression but low levels MUC1 at the cell surface. These results are not necessarily contradictory. Acute type ATL cells may acquire the cell surface expression of MUC1 during their transformation. Since the precise mechanism by which MUC1 is overexpressed in cancer cells remains unknown [52], ATL cells may serve as a model.

The aberrant overexpression of MUC1 has been thought to contribute to tumour progression by affecting cell adhesion and cell recognition [9]. The anti-adhesive properties of MUC1 inhibit cell-cell and cell-matrix contact and facilitate the migration and metastasis of tumour cells [41]. The anti-recognition properties of MUC1 allow tumour cells to evade immune surveillance. In the present study, we showed that the abrogation of MUC1 expression in KOB and KK1 cells using siRNA resulted in inhibition of cell proliferation. At the same time, we found that si-MUC1 cells caused a remarkable reduction in the number of clumps, which may be required for the proliferation of these cells. This suggests that MUC1 contributes to cell-cell adhesion. In fact, MUC1 has the potential to function as a receptor in cooperation with other adhesion molecules such as ICAM-1, E-selectin, and Siglecs [1; 53]. It is possible that some partner(s) forms a clump with MUC1 in these cells and plays a role in their

aggregative proliferation. MUC1-mediated cell aggregation is rarely observed [41; 54]. In this regard, hairy cell leukaemia (HCL) cells required the underexpression of Ras homolog gene family member H (RhoH) in order to proliferate [55]. They indicated that the reconstruction of RhoH in HCL cell lines resulted in a reduction in clumps and an attenuation of the decrease in non-aggregated cells, similar to the results of our si-MUC1 experiment. They further showed that cell aggregation and/or clumping is vital for malignant progression in HCL cells *in-vivo*. Therefore, MUC1 may play an important role in cell-cell adhesion, in forming clumps, and in the progression of ATL.

Recent findings indicate that MUC1 CT contributes to signal transduction, including the Wnt- β -catenin, p53, and NF- κ B pathways. This new concept helps to explain the mechanism of MUC1-mediated cancer cell growth [56; 57]. Another MUC1-mediated effect is resistance to apoptosis [42]. Recent studies suggest that MUC1 suppresses apoptosis through the regulation of JNK, NF- κ B, HSP90, and extrinsic apoptotic pathways [58]. It was also demonstrated that MUC1 CT binds directly to the p53 regulatory domain and suppressed the p53-dependent apoptotic response [59]. Our results showed no enhancement of Nutlin-3a-induced apoptosis by si-MUC1, however, we found that si-MUC1 enhanced TRAIL- and MG132-induced apoptosis in cells with both wild-type (KOB) and mutant (KK1) p53. These results indicate that MUC1 expression correlates with resistance to apoptosis in ATL cells, via a mechanism not entirely dependent on p53. Since TRAIL-induced apoptosis plays a role

in cancer immuno-surveillance [60], ATL cells may evade attack by using this anti-recognition property of MUC1.

In conclusion, we found MUC1 to be overexpressed in ATL cells and to have unique roles in cell-growth. In addition, MUC1 is also a potential prognostic marker of ATL. However, the *raison d'etre* of MUC1 in ATL cells remains enigmatic. There is little evidence for functions of MUC1 in haematological malignant cells compared with epithelial cancer cells. Although further investigation of MUC1 in haematological malignancies is required, ATL cells may become a good model, especially in molecular targeting therapy against MUC1.

Conflict-of-interest disclosure:

The authors declare that there are no competing financial interests related to this study.

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Legends

Figure.1 ATL cell lines have high levels of MUC1.

(A) Quantitative RT-PCR for MUC1 in various haematological cell lines. Data were normalized to the GAPDH level measured in the same sample. (B, C, and E) Flowcytometric analysis of the cell surface expression of MUC1 using anti-MUC1/CD227 (B and C), anti-MUC1/DF3, anti-MUC1/Core, or anti-MUC1/HMFG-1 (E) in ATL cell lines and other haematological cell lines. Shaded and unshaded peaks correspond to specific and control staining, respectively. RFI (the ratio of mean fluorescence intensity for specific staining to that for control staining) is indicated in each panel. (D) Representative photographs of immunocytochemistry. Cells were smeared on glass slides, fixed and stained with anti-MUC1/CD227 and Hoechst. Signals were fluoroscopically observed using Leica DM4000B Microsystems. The size bar corresponds to 10 μ m.

Figure. 2 Expression of MUC1 in primary ATL cells and correlation between MUC1 and prognosis.

(A) Quantitative RT-PCR for MUC1 in primary; 52 acute-type and 12 chronic-type, ATL cases and 10 healthy donors. All data were normalized to the GAPDH level measured in the same sample and were also analyzed using Student's t-test. p values are indicated. ** P<0.01. (B) Overall survival curves for ATL patients separated into those with high (n=30) and

low (n=30) MUC1 mRNA levels. The group with high MUC1 mRNA levels had significantly shorter survival times. Survival rates were analyzed using the Kaplan-Meier method, with the log rank test used to calculate the significance of differences. * $P < 0.05$. (C and D) Flowcytometric analysis of the cell surface expression of MUC1 in primary ATL cases. Samples from 17 acute-type ATL patients, 5 chronic-type ATL patients, and 5 healthy donors were investigated. (C) Representative data for normal CD4 lymphocytes and primary ATL cells are shown. Shaded and unshaded peaks correspond to specific and control staining, respectively. RFI is indicated in each panel. (D) Comparison of expression profiles between acute-type ATL patients, chronic-type ATL patients, and healthy donors. Analyses were performed using RFI and p values are indicated. * $P < 0.05$, ** $P < 0.01$.

Figure. 3 MUC1 contributes to cell proliferation in ATL cell lines.

(A) Quantitative RT-PCR for MUC1 in siRNA experiments using KOB and KK1 cells. MUC1 or control siRNA was transfected, cells were harvested at the indicated time points, and Quantitative RT-PCR was performed. The amount of mRNA in siMUC1 cells relative to that in si-control cells is indicated. (B) Cell surface expression of MUC1 in siRNA experiments. Twenty four hours after MUC1 or control siRNA was transfected, cells were harvested and FCM was performed using anti-MUC1/CD227. The shaded peak and bold line correspond to MUC1 expression in si-control cells and siMUC1 cells, respectively. The dotted

line corresponds to the negative control. (C) After MUC1 or control siRNA was transfected, cell proliferation was assessed by MTS assay at the indicated time points by comparing si-control cells with siMUC1 cells. Results are expressed as the ratio of siMUC1 cells to si-control cells. Data are the mean \pm SD from three independent experiments. ** P<0.01.

Figure. 4 MUC1 contributes to cell aggregation in ATL cell lines.

(A and B) Homotypic adhesion assays were performed in KOB and KK1 cells. MUC1 or control siRNA was transfected, and patterns of aggregation were analyzed at the indicated time points. Five representative fields were photographed. (A) Representative micrographs after 72 hours are shown. The size bar corresponds to 100 μ m. (B) The number of clumps and cells not in an aggregate was counted using these photographs. The sum of the number of clumps and non-aggregated cells was considered the total cell number. The number of non-aggregated cells was then divided by the total cell number. Data are the mean \pm SD for 5 different fields. ** P<0.01.

Figure. 5 MUC1 contributes to the inhibition of apoptosis in ATL cells.

Twenty four hours after MUC1 or control siRNA was transfected into KOB and KK1 cells, the cells were treated with 400ng/mL of TRAIL, 5 μ M of MG132, or 20 μ M of Nutlin-3a for another 24 hours. The cells were then harvested, and Annexin-V/PI staining was performed by FCM.

Annexin-V-positive cells were considered to be apoptotic cells. Data are the mean \pm SD for three independent experiments. ** P<0.01.

Figure. 1

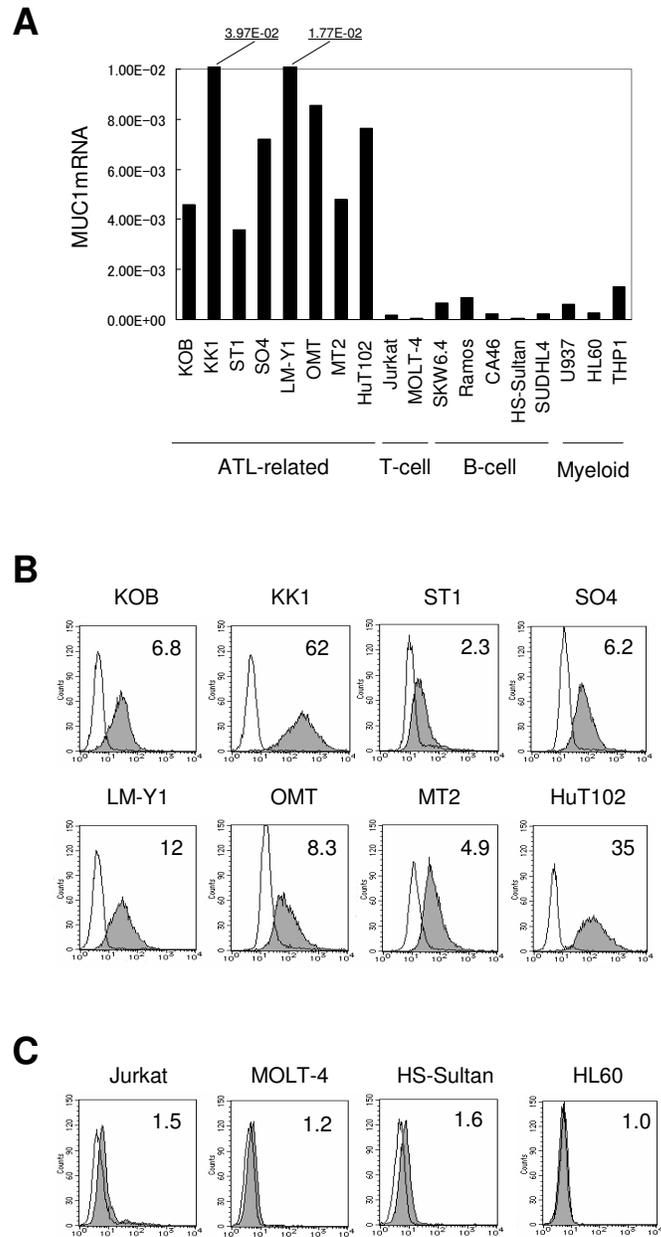
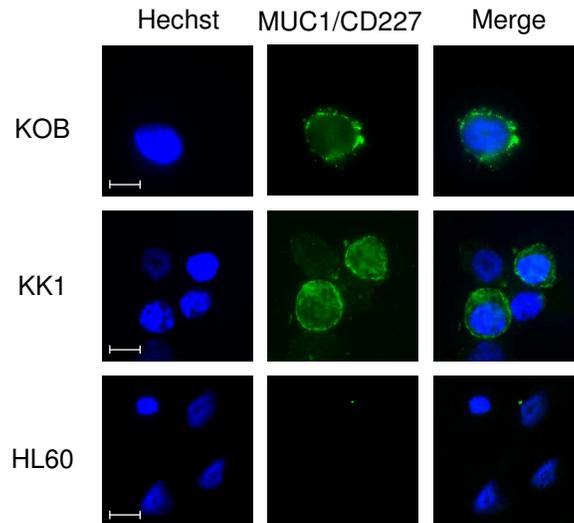


Figure. 1 (continued)

D



E

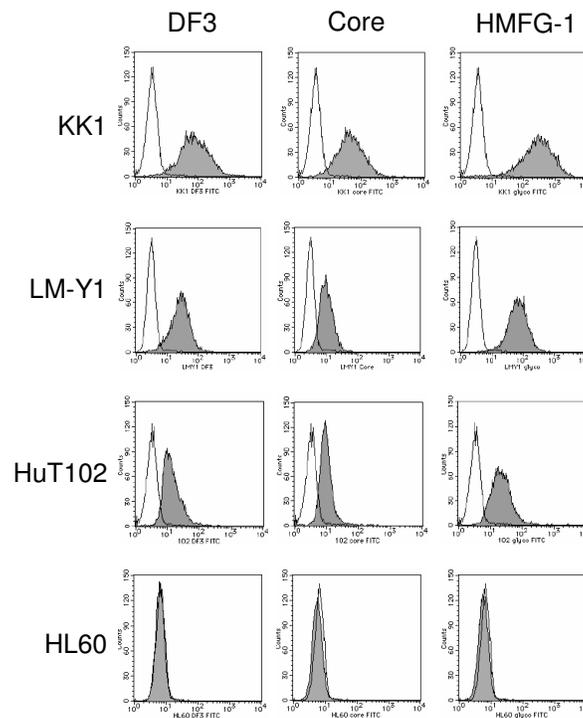


Figure. 2

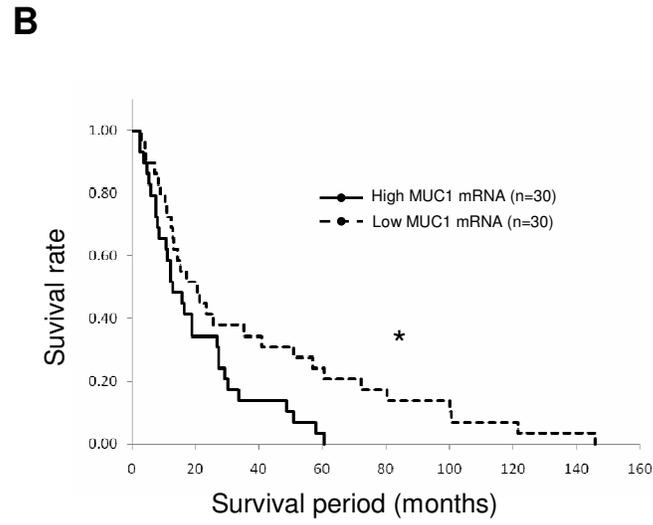
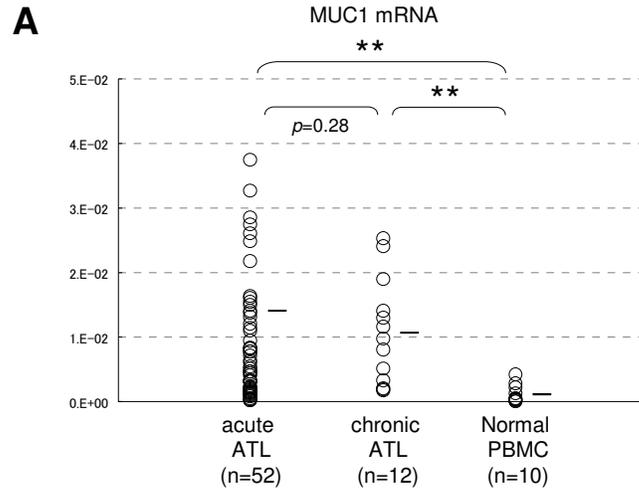
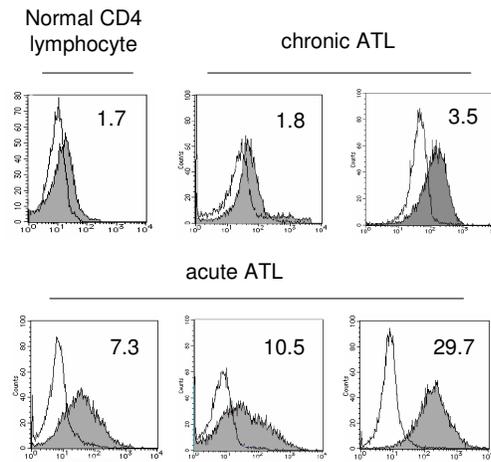


Figure. 2 (continued)

C



D

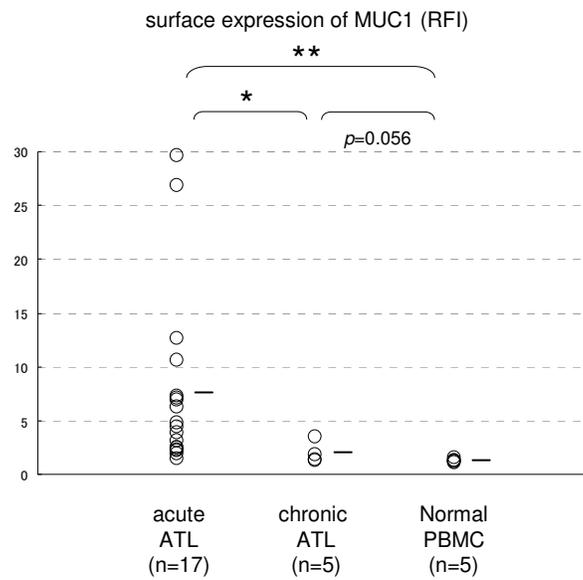


Figure. 3

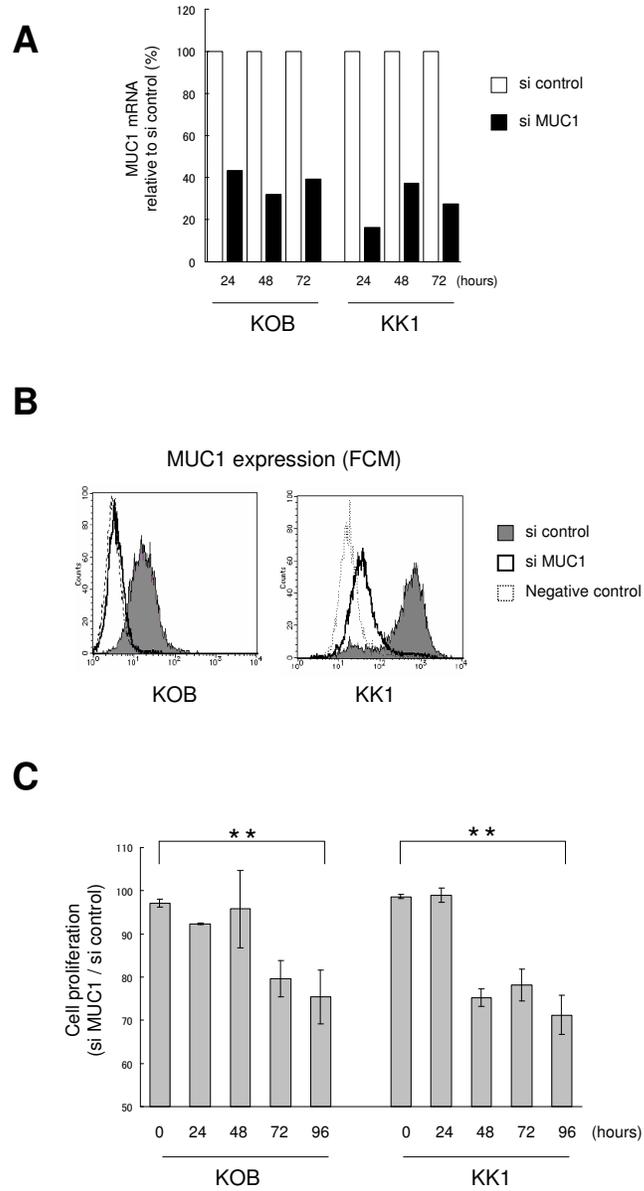


Figure. 4

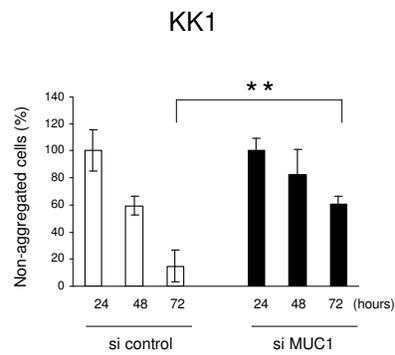
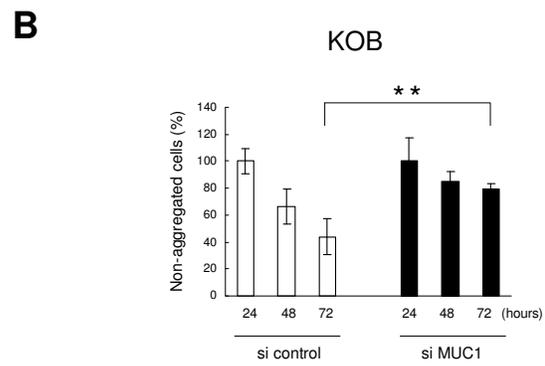
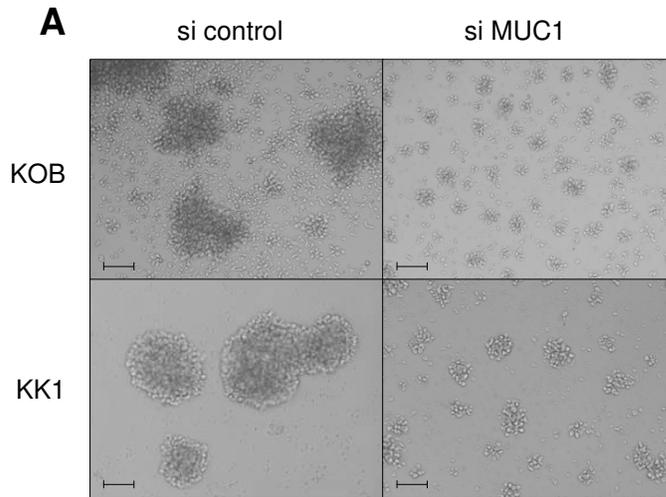


Figure. 5

