MUC1 Oncoprotein Regulates Bcr-Abl Stability and Pathogenesis in Chronic Myelogenous Leukemia Cells

Takeshi Kawano, Masaki Ito, Deepak Raina, Zekui Wu, Jacalyn Rosenblatt, David Avigan, Richard Stone, and Donald Kufe

Abstract

Chronic myelogenous leukemia (CML) results from expression of the Bcr-Abl fusion protein in hematopoietic stem cells. The MUC1 heterodimeric protein is aberrantly overexpressed in diverse human carcinomas. The present studies show that MUC1 is expressed in the human K562 and KU812 CML cell lines. The results show that MUC1 associates with Bcr-Abl through a direct interaction between the Bcr N-terminal region and the MUC1 cytoplasmic domain. Stable silencing of MUC1 decreased cytoplasmic Bcr-Abl levels by promoting Bcr-Abl degradation. Silencing MUC1 was also associated with decreases in K562 and KU812 cell self-renewal capacity and with a more differentiated erythroid phenotype. The results further show that silencing MUC1 increases sensitivity of CML cells to imatinib-induced apoptosis. Analysis of primary CML blasts confirmed that, as found with the CML cell lines, MUC1 blocks differentiation and the apoptotic response to imatinib treatment. These findings indicate that MUC1 stabilizes Bcr-Abl and contributes to the pathogenesis of CML cells by promoting self renewal and inhibiting differentiation and apoptosis. [Cancer Res 2007;67(24):11576–84]

Introduction

Chronic myelogenous leukemia (CML) is a disorder of the hematopoietic stem cell that is characterized by marked accumulation of cells within the granulocytic lineage. CML is caused by a t(9;22)(q34;q11) chromosomal translocation and expression of the p210 Bcr-Abl fusion protein. Expression of Bcr-Abl in hematopoietic cells confers independence from growth factors for proliferation and survival. The Bcr N-terminal coiled-coil oligomerization domain activates the Bcr-Abl kinase function and is of importance for Bcr-Abl-mediated transformation and leukemogenesis (1). The Ab1 SH2 domain also contributes to the malignant phenotype and a CML-like myeloproliferative disease in mice (2). Imatinib mesylate (STI571, Gleevec) is a potent inhibitor of Ab1 kinase activity that blocks the growth of Bcr-Abl–positive cells and induces cytogenetic responses in patients with CML (3, 4). Treatment with imatinib, however, can be associated with residual Bcr-Abl–positive cells as detected by reverse transcription–PCR (RT-PCR) (5), indicating that inhibition of Ab1 kinase activity may not be sufficient for eradication of the disease. In addition, CML cells can emerge with resistance to imatinib, often as a result of mutations in Bcr-Abl that disrupt binding of the drug (6).

The chronic phase of CML has a median duration of ~5 years before progression to blast crisis that is characterized by accumulation of blasts in the bone marrow, peripheral blood, and/or extramedullary tissues. The blast phase of CML has been attributed to the acquisition of genetic or epigenetic changes that block differentiation and increase self-renewal capacity (7). Progression of CML to the blast phase is associated with decreased responsiveness to imatinib and relapses to this agent due to Bcr-Abl point mutations or amplification of the Bcr-Abl gene (8). Isolation of granulocyte-macrophage progenitors from patients with CML in blast crisis and imatinib-resistant disease has shown increased nuclear levels of the Wnt pathway effector β-catenin (9). Suppression of β-catenin with axin decreased self-renewal capacity and leukemic potential of these cells, indicating that dysregulation of β-catenin contributes to development of the blast phase (9).

The MUC1 transmembrane glycoprotein is expressed by normal secretory epithelial cells (10) and hematopoietic cells of the lymphoid lineage (11, 12). Diverse human carcinomas aberrantly overexpress MUC1 (10). In addition, MUC1 has been detected at high levels in multiple myeloma (13) and non–Hodgkin’s lymphomas (14). MUC1 is synthesized as a single polypeptide that undergoes autocleavage into two subunits in the endoplasmic reticulum. The MUC1 N-terminal subunit (MUC1-N) contains variable numbers of 20 amino acid tandem repeats that are modified by O-linked glycans, a characteristic of the mucin-type glycoproteins (15). MUC1-N is tethered to the cell membrane through formation of a heterodimer with the transmembrane MUC1 C-terminal subunit (MUC1-C), which transduces signals to the interior of the cell (16). With transformation, the MUC1-C subunit accumulates in the cytosol and is targeted to the nucleus and the mitochondrial outer membrane (17–19). The MUC1-C cytoplasmic domain (CD) is phosphorylated by c-Src (20) and glycogen synthase kinase 3β (21). MUC1-C also binds directly to β-catenin and increases β-catenin levels by blocking its degradation (22). Other studies have shown that overexpression of MUC1 confers transformation (22, 23).

Recent work has shown that cytoplasmic MUC1-C functions as a c-Ab1 substrate and blocks nuclear targeting of c-Ab1 in the response to genotoxic stress (24). c-Ab1 contains three nuclear localization signals and a nuclear export signal in the carboxy terminal region that are responsible for shuttling c-Ab1 between the nucleus and cytoplasm (25). Nuclear c-Ab1 is activated by genotoxic agents and induces apoptosis (26, 27). Binding to 14-3-3 proteins sequesters c-Ab1 in the cytoplasm (28). In the response to DNA damage, c-Jun–NH2 kinase phosphorylates 14-3-3 (29) and releases c-Ab1 for targeting to the nucleus (28). The interaction between c-Ab1 and 14-3-3 is disrupted by direct binding of MUC1-C and c-Ab1 SH2 domain (24). However, in contrast to
14-3-3, c-Abl is not released from MUC1 in the response to DNA damage (24). As such, MUC1 retains c-Abl in the cytoplasm and thereby blocks the proapoptotic function of c-Abl in the nucleus (24). Treatment with imatinib promotes shuttling of Bcr-Abl from the cytoplasm to the nucleus (29). Moreover, entrapment of Bcr-Abl in the nucleus induces apoptosis (29). These findings suggested that, like c-Abl, MUC1-C could play a role in sequestering Bcr-Abl in the cytosol and thereby promote Bcr-Abl-mediated transformation.

The present studies show that MUC1-C associates with Bcr-Abl in CML cells. The MUC1-CD binds directly to the Bcr N-terminal region of Bcr-Abl and stabilizes Bcr-Abl. The results further show that MUC1 promotes the malignant CML cell phenotype.

**Materials and Methods**

**Cell culture.** Human K562 and KU812 CML and BC-1 lymphoma cells were cultured in RPMI 1640 (Cellgro) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Cellgro), 100 units/mL penicillin, 100 μg/mL streptomycin [American Type Culture Collection (ATCC)], and 2 mmol/L L-glutamine (ATCC). Human 293 cells were grown in DMEM with 10% FBS, antibiotics, and 1-glutamine. Cells were treated with imatinib (Novartis).

**Immunoprecipitation and immunoblotting.** Cells were lysed by sonication in the presence of 10 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 5 mmol/L EDTA, 0.5% NP40, 100 μg/mL phenylmethylsulfonyl fluoride, and protease inhibitor mixture. Soluble proteins were incubated with anti-MUC1-C (Ab5; Labvision), anti-Bcr (G6, N-20, C-20; Santa Cruz Biotechnology), or anti-c-Abl (Santa Cruz Biotechnology) for 2 h at 4°C.
followed by precipitation with protein A/G beads (Pierce). Immune complexes and lysates were subjected to immunoblot analysis with anti-MUC1-N (MAb DF3; ref. 10), anti-MUC1-C, anti-h-actin (Sigma), anti-glutathione S-transferase (GST; Oncogene Biosciences), anti-IkBα (Santa Cruz Biotechnology), and anti-lamin B (Oncogene Biosciences). Reactivity was detected with horseradish peroxidase–conjugated second antibodies and chemiluminescence (Amersham Biosciences).

**In vitro binding assays.** Purified GST, GST-MUC1-CD(1–72), GST-MUC1-CD(1–45), GST-MUC1-CD(46–72), GST-Bcr(1–197), GST-Bcr(1–385), or GST-Bcr(1–426) were immobilized on glutathione beads (Pierce). The beads were incubated with cell lysates, His-MUC1-CD(1–72), or His-Bcr(1–426) for 2 h at 4°C and washed, and the adsorbates were analyzed by immunoblotting. In certain binding experiments, purified MUC1-CD was first incubated in the absence and presence of recombinant truncated Abl (kinase domain; New England Biolabs) and 200 μM ATP for 30 min at 30°C as described (24).

**Silencing of MUC1 in CML cells.** The BLOCK-iT Target Screening System (Invitrogen) was used to generate small interfering RNAs (siRNA) that target two MUC1 sequences (#1, AAGGTACCATCAATGTCCACG or #2, AAGTTCAGTGCCCAGCTCTAC) and a control sequence (CGCTTACC-GATTCAGAATGG). The siRNA cassettes were transferred to pHAGE-fullEF1a-MCS-IZsGreen-W for the generation of lentiviral vectors. The K562 and KU812 cells were infected with the lentiviruses at a multiplicity of infection of 5 in the presence of 8 μg/mL polybrene (Sigma). Cell clones were selected in methylcellulose semisolid medium for expression of EGFP and assayed for down-regulation of MUC1 by immunoblotting. For construction of adenoviruses, the siRNAs that target MUC1 sequences #1 or #2 and a control sequence targeting the luciferase gene were ligated into the pSIREN-DNR vector (Clontech). The siRNA cassettes were transferred to pLP-Adeno-X-PRLS by Cre/LoxP-mediated recombination. The vectors were then packaged into adenoviruses by transfection into 293 cells. Cells were infected with recombinant viruses at a multiplicity of infection of 5.

**Subcellular fractionation.** Nuclear and cytoplasmic fractions were prepared using the nuclear extraction kit (Active Motif).

**RT-PCR.** Total cellular RNA was extracted with TRIZOL (Roche). Bcr-Abl–specific (Bcr forward, 5¶-TCAGACCCTGAGGCTCAAAGTC-3¶ and Abl reverse, 5¶-GGAGCTGCAGATGCTGACCACC-3¶), MUC1-specific (forward, 5¶-GGTACCATCAATGTCCACG-3¶ and reverse, 5¶-CTACAAGTTGGCA-GAAGTGG-3¶-3¶-3¶) primers (Invitrogen) were used for reverse transcription and amplification (GeneAmp PCR System; Perkin-Elmer Applied Systems). Amplified fragments were analyzed by electrophoresis in 2% agarose gels.

**Pulse-chase analysis.** Cells were cultured in methionine-free medium containing 250 μCi/mL [35S]-labeled methionine (EasyTag Protein Labeling Mix; Perkin-Elmer) for 2 h, washed, and then chased in the presence of complete medium. Anti-Bcr precipitates were subjected to SDS-PAGE and autoradiography as described (22). Intensity of the signals was determined by densitometric scanning.

**Analysis of self-renewal capacity.** Cells were seeded at 2,000 per well of a six-well plate containing 0.4% methylcellulose in RPMI 1640 supplemented with 10% or 2% FBS. At 4 weeks, colonies were counted under a stereomicroscope.

**Apoptosis assays.** Cells were fixed in 70% ethanol and incubated in PBS containing 50 μg/mL RNase and 2.5 μg/mL propidium iodide. DNA content was analyzed by flow cytometry. The percentage of cells with sub-G1 DNA

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**Figure 2.** MUC1-CD binds directly to Bcr. A, lysates from BC-1 cells were precipitated with control mouse IgG, anti-Bcr(N-20), or anti-Bcr(C-20). The precipitates were immunoblotted with the indicated antibodies. B, lysate from BC-1 cells was incubated with GST or GST–MUC1-CD. The adsorbates were immunoblotted with the indicated antibodies. Whole cell lysate was included as a control. C and D, purified His-MUC1-CD was incubated with GST or the indicated GST-Bcr fusion proteins (C). His-Bcr(1–426) was incubated with GST or the indicated GST-MUC1-CD fusion proteins (D). The adsorbates were immunoblotted with anti-His. Input of the GST and GST–fusion proteins was assessed by immunoblotting with anti-GST.
was determined by the MODFIT LT program (Verity Software). Primary CML blasts were stained with Hoechst dye (Sigma) as described (30) and visualized under a fluorescence microscope for condensed and fragmented nuclei. Five hundred cells were scored for apoptosis in each of three independent experiments.

Isolation of mononuclear cells from bone marrows of patients with CML. Bone marrow aspirates were obtained from patients with chronic phase or blast crisis CML in accordance with a protocol approved by the Institutional Review Board. Mononuclear cells were isolated by Ficoll density centrifugation. Certain chronic phase CML samples were subjected to positive CD34 selection using the MiniMacs CD34 cell isolation kit (Miltenyi Biotec). Bone marrow mononuclear cells isolated from two patients with CML in blast crisis were cultured for 7 days in RPMI 1640 with 15% FBS and antibiotics.

Fluorescence in situ hybridization. Cytospin preparations of mononuclear cells isolated from the bone marrow of a patient with CML in blast crisis were stained with anti-MUC1-N using the Vectastain ABC kit (Vector Laboratories). Destaining was performed according to the manufacturer’s recommendations (BioView, Inc.). The slides were then dehydrated in ethanol. Denaturation of the sample and probes (LSI Bcr-Abl Dual Color, Dual Fusion Translocation Probe; Vysis) was completed at 73°C for 2 min, and the samples were hybridized with the probes at 37°C for 48 h. The samples were washed, counterstained with Blue View Drop (BioView), and visualized under a fluorescence microscope.

Results

MUC1-C associates with Bcr-Abl in CML cells. MUC1 is expressed in epithelial cells as a heterodimer of the MUC1-N and MUC1-C subunits. To assess expression of MUC1 in K562 CML cells, lysates were immunoblotted with antibodies against MUC1-N and MUC1-C. The results show that MUC1-N is expressed as the high molecular weight (>250 kDa) glycosylated form as detected by MAβ DF3 (Fig. 1A). The transmembrane MUC1-C subunit was detectable as ~25 to 15 kDa species (Fig. 1A), consistent with that found in carcinoma cells (31). Similar patterns of MUC1 expression were found by probing lysates of KU812 CML cells (Fig. 1A). Recent studies have shown that MUC1-C associates with c-Abl and blocks nuclear targeting of c-Abl in the response to DNA damage (24). Immunoblot analysis of anti–MUC1-C precipitates with anti-Bcr showed that MUC1-C associates with the p210 Bcr-Abl fusion protein in both K562 and KU812 cells (Fig. 1B). In the reciprocal experiment, immunoblot analysis of anti-Bcr and anti–c-Abl precipitates with anti-MUC1-C supported the association of Bcr-Abl and MUC1-C (Fig. 1C). Recent studies have shown that MUC1-C associates with c-Abl and blocks nuclear targeting of c-Abl in the response to DNA damage (24).

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MUC1-CD binds directly to Bcr. To determine if MUC1-CD associates with Bcr, we studied human BC-1 lymphoma cells that express endogenous MUC1 and Bcr, but not Bcr-Abl. Immunoprecipitation of Bcr from BC-1 lysates with two different anti-Bcr antibodies showed that Bcr associates with endogenous MUC1-C (Fig. 2A). To determine if MUC1-CD associates with Bcr, BC-1 lysates were incubated with GST or GST–MUC1-CD. Analysis of the adsorbates by immunoblotting with anti-Bcr showed binding of MUC1-CD to the 160 and 130 kDa forms of Bcr (Fig. 2B). The human Bcr protein contains 1,271 amino acids, of which the N-terminal 1 to 902/927 amino acids are expressed in the p210 Bcr-Abl protein (32). To determine whether MUC1-CD binds to the N-terminal region of Bcr, we incubated His–MUC1-CD with GST or GST–Bcr fusion proteins generated from the N-terminal 197, 385 and 426 amino acids (Fig. 2C). The results show that MUC1-CD binds to Bcr(1–385) and Bcr(1–426), and not Bcr(1–197) (Fig. 2C). These findings indicate that MUC1-CD(1–45) binds directly to the Bcr N-terminal region (amino acids 197–426).

MUC1-CD interacts with Bcr and Abl in Bcr-Abl. The finding that MUC1-CD(1–45) binds to Bcr, and the previous demonstration that the c-Abl SH2 domain interacts with the MUC1-CD phosphorylated Tyr60 site (24) suggested that MUC1-CD may bind to both Bcr and Abl in Bcr-Abl. To address this possibility, 293 cells were transfected to express Bcr-Abl, GFP-tagged MUC1-CD(46–72), and Flag-tagged MUC1-CD. Immunoblot analysis of anti-Bcr precipitates with anti-MUC1-C showed that Bcr-Abl associates with GFP–MUC1-CD(46–72) (Supplementary Fig. S1A). As a control, Bcr-Abl was also found to associate with Flag–MUC1-CD (Supplementary Fig. S1A). These results indicate that, in addition to MUC1-CD(1–45) as shown above, the interaction between Bcr-Abl and MUC1-CD can also be mediated by MUC1-CD(46–72). To determine whether MUC1-CD binds to both Bcr and Abl, we incubated purified MUC1-CD with GST–c-Abl SH2 and His-Bcr(1–426) in vitro. Whereas the interaction between MUC1-CD and c-Abl SH2 is dependent on c-Abl phosphorylation of MUC1-CD on Tyr60 (24), we used MUC1-CD without and with phosphorylation by recombinant Abl kinase and ATP (Supplementary Fig. S1B). Analysis of adsorbates to glutathione beads with anti-Bcr and anti–MUC1-C showed that phosphorylated, and not unphosphorylated, MUC1-CD is pulled down by GST–c-Abl SH2 in a complex with Bcr(1–426) (Supplementary Fig. S1B, lanes 4 and 6). Among other controls, there was no detectable binding of GST–c-Abl SH2 with Bcr(1–426) (Supplementary Fig. S1B, lane 3). These findings collectively indicate that MUC1-CD binds to both Bcr and Abl in Bcr-Abl.

MUC1 stabilizes the Bcr-Abl protein. To address the significance of the MUC1-C–Bcr-Abl interaction, we infected K562 cells with a lentivirus expressing a siRNA that targets the MUC1 sequence 5’-AAGGTACCATCAATGTCCACG-3’ (MUC1-siRNA#1) in MUC1-C. Compared with wild-type K562 cells and those infected with a lentivirus expressing a control siRNA (CsiRNA), MUC1 expression was down-regulated by MUC1-siRNA#1.
To avoid potential off-target effects, we also infected K562 cells with a lentivirus expressing a siRNA that targets a different MUC1 sequence (5'-AAGTTCAGTGCCCAGCTCTAC-3'; MUC1siRNA#2) in MUC1-N. Down-regulation of MUC1-C was similar with the two MUC1siRNAs (Fig. 3A, left). Notably, silencing MUC1 was associated with a partial decrease in Bcr-Abl levels (Fig. 3A, left). Analysis of cytoplasmic and nuclear fractions further showed that the down-regulation of Bcr-Abl is detectable in the cytoplasm and that silencing MUC1 is not associated with increases in nuclear Bcr-Abl (Fig. 3A, right). Immunoblotting with antibodies against the cytosolic IκBα and nuclear lamin B proteins was used to confirm equal loading of the lanes and purity of the fractions (Fig. 3A, right). Silencing of MUC1 in KU812 cells was also associated with partial down-regulation of Bcr-Abl expression (Fig. 3B, left). Moreover, the decreases in Bcr-Abl were detectable in the cytosol and not related to targeting of Bcr-Abl to the nucleus (Fig. 3B, right). These results indicate that MUC1 functions in the up-regulation of Bcr-Abl expression. To determine whether MUC1 affects Bcr-Abl transcription, we analyzed Bcr-Abl expression by RT-PCR. The results indicate that silencing MUC1 has little, if any, effect on Bcr-Abl mRNA levels in both K562 and KU812 cells (Supplementary Fig. S2). Stability of the Bcr-Abl protein was therefore assessed by pulse-chase labeling and precipitation of Bcr-Abl with anti-Bcr. Autoradiography of the Bcr-Abl signals indicated that silencing of MUC1 decreases Bcr-Abl stability in K562 cells (Fig. 3C, left). Analysis of three separate experiments showed that Bcr-Abl has a half-life of 38 and 21 h in the presence and absence of MUC1, respectively (Fig. 3C, right). Silencing MUC1 in KU812 cells was also associated with a decrease in Bcr-Abl stability (Fig. 3D, left). The half-lives of Bcr-Abl in the KU812/CsiRNA and KU812/MUC1siRNA cells were 38 and 17 h, respectively (Fig. 3D, right). These findings indicate that MUC1 increases Bcr-Abl expression by stabilizing the Bcr-Abl protein.

**Silencing MUC1 decreases self-renewal capacity.** To determine if MUC1 affects CML cell proliferation, the K562 cells were grown in medium containing 10% or 2% FBS. The results show that silencing MUC1 has little effect on K562 cell growth in 10% FBS (Supplementary Fig. S3A). In addition, K562/MUC1siRNA cell growth was modestly attenuated compared with that of K562/CsiRNA cells in 2% FBS (Supplementary Fig. S3A). By contrast, self-renewal capacity as determined by colony formation in methylcellulose was substantially decreased by silencing MUC1 in K562 cells.
K562 cells cultured in the presence of 10% and 2% FBS (Fig. 4A–C). Silencing MUC1 also had a modest effect on growth of KU812 cells in 2% FBS (Supplementary Fig. S3B). However, as found with K562 cells, self-renewal capacity of KU812 cells was significantly decreased by silencing MUC1 (Fig. 4D).

Silencing MUC1 is associated with a differentiated phenotype. K562 cells respond to hemin and certain differentiating agents with the induction of hemoglobin synthesis (33, 34). Staining of K562 and K562/CsiRNA cells with benzidine to assess hemoglobin production showed a low percentage of positive cells (Fig. 5A). By contrast, silencing MUC1 was associated with an increase in K562 cells that stain positively with benzidine (Fig. 5A). Similar results were obtained with KU812 cells silenced for MUC1 (Supplementary Fig. S4). Quantitation of benzidine staining showed that silencing MUC1 significantly increases production of hemoglobin in both K562 and KU812 cells (Fig. 5B). To confirm that MUC1 is responsible for these observations, we infected K562 cells with adenoviruses expressing a control CsiRNA, MUC1siRNA#1, or MUC1siRNA#2 (Fig. 5C). The results show that down-regulation of MUC1 is associated with increases in benzidine staining (Fig. 5D). These findings indicate that MUC1 suppresses differentiation of K562 and KU812 cells.

Silencing MUC1 increases sensitivity to imatinib. Previous work in carcinoma cells has shown that MUC1 blocks the apoptotic response to genotoxic anticancer agents (17). However, the role of MUC1 in regulating apoptosis of malignant hematopoietic cells has not been studied. To explore this issue, we treated the K562 and KU812 cells with imatinib and assayed for the induction of apoptosis by sub-G1 DNA content. The results show that silencing MUC1 is associated with imatinib-induced increases in K562 (Supplementary Fig. S5A) and KU812 (Supplementary Fig. S5B) cells with sub-G1 DNA content. These findings were confirmed in additional experiments (Supplementary Fig. S5C), indicating that MUC1 attenuates the apoptotic response to imatinib treatment.
Function of MUC1 in primary CML blasts. Mononuclear cells prepared from the bone marrow of a patient with CML in blast crisis were studied for MUC1-N expression by immunostaining and for Bcr-Abl by cytogenetics (Fig. 6A). MUC1-N was detectable in Bcr-Abl–positive cells, indicating that MUC1 is expressed by CML blasts (Fig. 6A). Moreover, analysis of anti–MUC1-C precipitates from lysates of primary CML blasts showed that MUC1-C associates with Bcr-Abl (Fig. 6B). By contrast, there was no detectable expression of MUC1 in mononuclear cells or CD34–selected cells prepared from five patients with chronic phase CML (data not shown). Primary CML blasts from two patients were cultured for 7 days. Infection of the cells with adenoviruses expressing MUC1siRNA1 or MUC1siRNA2 was associated with down-regulation of MUC1-C expression (Fig. 6C). Previous work showed involvement of the erythroid lineage in CML blasts from ~40% of patients (35). In this regard and as found for K562 and KU812 cells, silencing MUC1 in the CML blasts resulted in increased hemoglobin production (Supplementary Fig. S6A). Analysis of the CML blasts silenced for MUC1 with MUC1siRNA1 or MUC1siRNA2 gave similar increases in benzidine-positive staining (Fig. 6D). The CML blasts were also treated with imatinib, stained with Hoechst dye, and monitored for nuclear condensation and fragmentation as a measure of apoptosis (Supplementary Fig. S6B). Silencing MUC1 was associated with an increased apoptotic response to imatinib treatment compared with that found in CML blasts infected with the control adenovirus (Supplementary Fig. S6B and C). These results indicate that, as found for the CML cell lines, MUC1 suppresses differentiation and apoptosis of primary CML blasts.

Discussion

MUC1-C associates with Bcr-Abl. Recent work has shown that MUC1-C interacts directly with c-Abl and sequesters c-Abl in the cytoplasm (24). Phosphorylation of MUC1-CD on Tyr 60 functions as a binding motif for the c-Abl SH2 domain (24). The present studies were performed to determine if MUC1 is expressed in CML cells and, if so, whether MUC1-C interacts with Bcr-Abl. Our results show that MUC1 is expressed in both CML cell lines and primary CML blasts and that MUC1-C associates with Bcr-Abl. We found that MUC1-C associates with the Bcr N-terminal region (amino acids 198–426) that is fused with Abl. In concert with these results, we show that MUC1-C also interacts with the endogenous Bcr p160 and p130 proteins. Bcr is a serine/threonine kinase that has a Dbl/CDC24 guanine nucleotide exchange factor function, a pleckstrin homology domain, and a RAC GTPase-activating protein domain (36). As found for MUC1-C (20–22), Bcr binds to β-catenin and regulates the Wnt signaling pathway (37). In addition, both MUC1-CD at Tyr 60 and the Bcr N-terminal region at Tyr 177 function as binding motifs for the Grb2 SH2 domain and thereby form complexes with the SOS exchange factor that regulates Ras (38–40). The MUC1-CD consists of 72 amino acids, of which the N-terminal 45 amino acids confer binding to Bcr and Bcr-Abl. Our results further indicate that, when phosphorylated, MUC1-CD Tyr 60 can function as a bridge from the Bcr N-terminal region to the Abl SH2 domain in Bcr-Abl. However, MUC1-C Tyr 60 is not required for binding to Bcr-Abl (data not shown), consistent with the interaction between MUC1-CD and Bcr. Bcr is a negative regulator of Bcr-Abl by mechanisms that are unclear (41, 42). In this context, Bcr could compete with Bcr-Abl for binding to MUC1-C in CML cells.

MUC1-C regulates stability of Bcr-Abl. MUC1-C interacts with diverse signaling molecules that include growth factor receptors (epidermal growth factor receptor, ErbB2-4, FGFR3; refs. 31, 43, 44), kinases (Src, c-Abl, Lyn, SYK3/5, PKCδ; refs. 20, 21, 24), and transcription factors (β-catenin, p53, ERα; refs. 18, 22, 45). The available evidence indicates that MUC1-C functions as a chaperone that holds proteins in certain configurations that regulate stability or activity. In this regard, MUC1-C destabilizes p53 and increases stability and activity of β-catenin and ERα (18, 22, 45). The present work shows that silencing MUC1 in CML cells is associated with down-regulation of Bcr-Abl expression. MUC1 had no effect on Bcr-Abl mrna levels. By contrast, silencing MUC1 decreased stability of the Bcr-Abl protein. Silencing MUC1 also decreased stability of the endogenous Bcr p160 and p130 proteins (data not shown), indicating that the effects of MUC1-C on Bcr-Abl stability may be mediated through binding of MUC1-C to the Bcr N-terminal region. In this regard, MUC1 has no apparent effect on stability of the c-Abl protein (24). Previous work has shown that Bcr-Abl is stabilized by HSP90 and that treatment of CML cells with geldanamycin or its analogues results in degradation of Bcr-Abl by the 26S proteosome (46, 47). MUC1-C also binds directly to HSP90, and this interaction contributes to intracellular targeting and not stabilization of MUC1-C (19). In contrast to Bcr-Abl, HSP90 binds to the MUC1-CD C-terminal region (amino acids 46–72; ref. 19). Thus, MUC1-CD could function in the formation of trimeric complexes of HSP90–MUC1-C–Bcr-Abl and thereby the stabilization of Bcr-Abl.

MUC1-C promotes self-renewal and blocks differentiation of CML cells. To define the biological effects of the MUC1-C–Bcr-Abl interaction, we asked if MUC1 affects growth and self-renewal of CML cells. Silencing MUC1 had little if any effect on K562 and KU812 cell growth in 10% FBS and was associated with a modest decrease in growth rate in the presence of 2% FBS. However, more pronounced decreases in self-renewal capacity were observed in both K562 and KU812 cells silenced for MUC1. One explanation for these findings is that silencing MUC1 results in decreases in Bcr-Abl levels that in turn would be less effective in supporting self renewal. Moreover, silencing MUC1 could have an effect independent of the MUC1-C interaction with Bcr-Abl. In this regard, recent work has shown that progression of CML is supported by self-renewing leukemic granulocyte-macrophage progenitors that exhibit activation of the β-catenin pathway (9). These results indicate that β-catenin contributes to conversion of chronic phase CML to blast crisis. Other work has shown that Bcr-Abl stabilizes β-catenin by a mechanism involving its tyrosine phosphorylation (48). Notably, MUC1-C also stabilizes β-catenin by directly blocking function of the β-catenin destruction complex (22) and therefore may play a role in activation of the β-catenin pathway in CML cells. Our results also show that silencing MUC1 in the CML cell lines and primary CML blasts is associated with the appearance of a more differentiated erythroid phenotype. These findings may be also attributable to the MUC1-dependent decreases in Bcr-Abl levels and thereby release of a Bcr-Abl–mediated block in differentiation. For example, destabilization of Bcr-Abl with HSP90 inhibitors induces erythroid differentiation of K562 cells. In addition, treatment of K562 cells with agents, such as 1-α-arabinofuranosylcytosine (ara-C), that decrease self-renewal results in the irreversible induction of hemoglobin synthesis (34). Thus, the decrease in self-renewal capacity associated with MUC1 silencing may contribute in part to the increase in hemoglobin production.

MUC1-C blocks the apoptotic response of CML cells to imatinib. Overexpression of MUC1 in human carcinoma cells
blocks the apoptotic response to gentoic anticancer agents, oxidative stress, and hypoxia (17, 49, 50). MUC1C is targeted to the mitochondrial outer membrane by an HSP90-dependent mechanism and attenuates activation of the intrinsic apoptotic pathway (17, 19). Inhibition of Bcr-Abl with imatinib induces apoptosis of BcraAbl–positive CML cells and is highly effective in treating patients with CML (3). The persistence of Bcr-Abl–positive cells in CML patients treated with imatinib has further indicated that factors other than inhibition of the Abl kinase function may be associated with the pathogenesis of CML (32). The present results indicate that silencing MUC1 in K562 and KU812 cells increases sensitivity to imatinib-induced apoptosis. Silencing MUC1 was also associated with an increase in ara-C–induced apoptosis (data not shown). Destabilization of Bcr-Abl with HSP90 inhibitors sensitizes CML cells to the induction of apoptosis through the intrinsic mitochondrial pathway. Thus, silencing MUC1 and thereby destabilization of Bcr-Abl could contribute to the increased sensitivity to imatinib. Moreover, based on studies in carcinoma cells, the increased sensitivity to imatinib could also be due in part to release of MUC1C–induced stabilization of the mitochondrial permeability transition and block in release of apoptogenic factors (17). Our results further show that MUC1 blocks the apoptotic response of primary CML blasts to imatinib treatment. These findings and the demonstration that MUC1 blocks differentiation of primary CML blasts indicate that MUC1 may be of importance to the pathogenesis of CML in patients.

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Takeshi Kawano, Masaki Ito, Deepak Raina, et al.