Direct Targeting of the Mucin 1 Oncoprotein Blocks Survival and Tumorigenicity of Human Breast Carcinoma Cells

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Abstract
The mucin 1 (MUC1) oncoprotein is aberrantly overexpressed by ~90% of human breast cancers. However, there are no effective agents that directly inhibit MUC1 and induce death of breast cancer cells. We have synthesized a MUC1 inhibitor (called GO-201) that binds to the MUC1 cytoplasmic domain and blocks the formation of MUC1 oligomers in cells, GO-201, and not an altered version, attenuates targeting of MUC1 to the nucleus of human breast cancer cells, disrupts redox balance, and activates the DNA damage response. GO-201 also arrests growth and induces necrotic death. By contrast, the MUC1 inhibitor has no effect on cells null for MUC1 expression or nonmalignant mammary epithelial cells. Administration of GO-201 to nude mice bearing human breast tumor xenografts was associated with loss of tumorigenicity and extensive necrosis, which results in prolonged regression of tumor growth. These findings show that targeting the MUC1 oncoprotein is effective in inducing death of human breast cancer cells in vitro and in tumor models. [Cancer Res 2009;69(12):5133–41]

Introduction
Mucins are extensively O-glycosylated proteins that are predominantly expressed by epithelial cells. The secreted and membrane-bound mucins form a physical barrier that protects the apical borders of epithelial cells from damage induced by toxins, microorganisms, and other forms of stress that occur at the interface with the external environment. The transmembrane mucin 1 (MUC1) has no sequence similarity with other membrane-bound mucins, except for the presence of a sea urchin sperm protein-esterokinase-agrin (SEA) domain (1). MUC1 is translated as a single polypeptide and then undergoes autocleavage at the SEA domain with the generation of two subunits that form a stable heterodimer (2, 3). The MUC1 NH2-terminal subunit (MUC1-N) contains variable numbers of tandem repeats that are modified by O-glycosylation (4). MUC1-N extends beyond the glycocalyx of the cell and is tethered to the cell surface through noncovalent binding to the transmembrane MUC1 COOH-terminal subunit (MUC1-C; ref. 5). MUC1-C consists of a 58-amino acid extracellular domain, a 28-amino acid transmembrane domain, and a 72-amino acid cytoplasmic domain that interacts with diverse signaling molecules (6). Shedding of MUC1-N into the protective physical barrier leaves MUC1-C at the cell surface as a putative receptor to transduce intracellular signals that confer growth and survival (7, 8).

With transformation and loss of polarity, MUC1 is expressed at high levels on the entire cell surface in a wide range of carcinomas of the breast, lung, prostate, gastrointestinal tract, and other epithelia (9). Loss of restriction to the apical membrane allows for the formation of complexes with the epidermal growth factor receptor (EGFR) and coactivation of EGFR-mediated signaling (7, 10). Overexpression of MUC1 by carcinoma cells is associated with the accumulation of MUC1-C in the cytosol and targeting of this subunit to the nucleus (11–13) and mitochondria (14, 15). Importantly, the MUC1 cytoplasmic domain (MUC1-CD) activates expression of gene signatures that are predictive of both response to tamoxifen and overall survival in breast cancer patients (16, 17). In this context, oligomerization of MUC1-C is necessary for its nuclear targeting and interaction with diverse effectors (18). For example, MUC1-CD functions as a substrate for c-Src (19), c-Abl (20), protein kinase Cδ (21), and glycogen synthase kinase 3β (22) and interacts directly with the Wnt pathway effector β-catenin (23, 24) and the p53 tumor suppressor (25). Other work has shown that overexpression of the MUC1 heterodimer confers anchorage-independent growth and tumorigenicity (12, 14, 25, 26), at least in part through stabilization of β-catenin (24). Moreover, consistent with a survival function for normal epithelial cells, overexpression of the MUC1 heterodimer confers resistance of carcinoma cells to stress-induced apoptosis by a mechanism mediated in part through suppression of intracellular reactive oxygen species (ROS; refs. 14, 27–29).

The present results show that targeting MUC1-C oligomerization blocks nuclear localization of MUC1-C and induces growth arrest and death of human breast cancer cells. The findings also show that inhibiting MUC1-C is highly effective in the treatment of human breast tumor xenografts in nude mice.

Materials and Methods

Cell culture. Human ZR-75-1 and MDA-MB-231 cell lines were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS), 100 units/ml penicillin, and 100 μg/ml streptomycin. Human MCF-7 breast cancer cells and 293 cells were grown in DMEM with 10% HI-FBS, antibiotics, and 2 mmol/L L-glutamine. Primary human breast cancer cells isolated from a malignant pleural effusion were cultured in RPMI 1640 containing 10% serum. Human MCF-10A breast epithelial cells were grown in mammary epithelial cell growth medium (Lonza). Cells were treated with GO-201, GO-202, or CP-1 peptides synthesized by MIT Biopolymer Laboratory and AnaSpec, Inc. Viability was determined by trypan blue exclusion.

Analysis of cell cycle distribution, apoptosis, and necrosis. Cells were harvested, washed with PBS, fixed with 80% ethanol, and incubated in PBS containing 40 μg/mL RNase and 40 μg/mL propidium iodide for 30 min at 37°C. Cell cycle distribution and sub-G1 DNA content were
determined by flow cytometry. For assessment of cell membrane integrity, cells were incubated with 1 μg/ml propidium iodide/PBS for 5 min at room temperature and then monitored by flow cytometry as described (29, 30). In the absence of other markers of apoptosis, uptake of propidium iodide reflects loss of membrane integrity indicative of necrosis (30).

**Human breast tumor xenograft models.** BALB/c nu/nu female mice (Charles River Laboratories) were implanted s.c. with 17β-estradiol plugs (0.72 mg Innovative Research) using a trocar gun. After 24 h, 1 × 10^7 ZR-75-1 or MCF-7 cells were injected s.c. in the flank. MDA-MB-231 cells (1 × 10^7) were similarly injected into mice without estradiol implants. When tumors were detectable, the mice were pair matched into treatment and control groups. Mice bearing tumors that were not within 10% of the mean volume were not included in the treatment and control groups. Each group contained 5 to 10 mice, each of which was ear tagged and followed throughout the study. Initial dosing was given at the time of pair matching (day 1). PBS (vehicle), GO-201, GO-202, and CP-1 were given daily by i.p. injection. Mice were weighed twice weekly. Tumor volume (V) was calculated using the formula V = L^2 × W/2, wherein L and W are the larger and smaller diameters, respectively.

**Results**

**Effects of GO-201 on MUC1 oligomer formation.** The MUC1-CD contains a CQC motif, which is necessary for the formation of oligomers (18). To determine whether MUC1 oligomerization is druggable, we synthesized a peptide derived from the NH2 terminal region of MUC1-CD, which contains the CQC motif (GO-201; Fig. 1A). A poly d-arginine transduction domain was included in the synthesis to facilitate the entry of the peptide into the cells (Fig. 1A; ref. 31). As a control, a similar peptide was synthesized in which the CQC motif was altered to AQA (CP-1; Fig. 1A). To assess binding of the peptides to MUC1-CD, we immobilized His-tagged MUC1-CD to a Biacore sensor chip. GO-201 bound to His-MUC1-CD with a dissociation constant (Kd) of 30 mM/L (Fig. 1B), which is similar to that obtained for binding of full-length MUC1-CD dimers (18). By contrast, there was no apparent binding of CP-1 (data not shown). Purified His-tagged MUC1-CD forms oligomers as detected by electrophoresis in polyacrylamide gels (Fig. 1C). Incubation of His-MUC1-CD with GO-201 substantially decreased oligomer formation with an increase in monomers (Fig. 1C). Moreover, incubation with CP-1 had little effect (Fig. 1C). To assess effects on MUC1 oligomerization in vivo, 293 cells were transfected with vectors expressing GFP-MUC1-CD and Flag-MUC1-CD (Fig. 1D, left). Complexes of GFP-MUC1-CD and Flag-MUC1-CD were detectable by coprecipitation of lysates from cells not exposed to GO-201 (Fig. 1D, right). In concert with the in vitro results, incubation of the transfected 293 cells with GO-201 was associated with the disruption of the interaction between Flag-MUC1-CD and GFP-MUC1-CD (Fig. 1D, right). In addition, CP-1 had no apparent effect (Fig. 1D, right). These results indicate that GO-201 binds to MUC1-CD and blocks formation of MUC1-CD oligomers in vitro and in cells.

**GO-201 disrupts MUC1-C function.** To assess peptide uptake, ZR-75-1 cells were incubated with 5 μmol/L FITC-labeled GO-201 (Fig. 2A). At 2 hours, analysis of the cells by flow cytometry showed a substantial increase in fluorescence intensity with a mean fluorescence index (MFI) of 145 (Fig. 2A). Further increases in MFI were identified at 6 and 24 hours (Fig. 2A). Treatment of ZR-75-1 cells with 5 μmol/L GO-201 or CP-1 for 3 days had no effect on cellular MUC1-C levels (Fig. 2B). However, in concert with effects on oligomerization, treatment with GO-201, and not CP-1, was associated with decreases in nuclear MUC1-C (Fig. 2B). Down-regulation of nuclear MUC1-C levels was also observed in the response of MCF-7 cells to GO-201 treatment (Supplementary Fig. S1A). Previous work has shown that MUC1-C decreases intracellular ROS levels (27–29). To determine whether targeting of MUC1-C with GO-201 disrupts redox balance, we incubated GO-201–treated ZR-75-1 cells with c-H2DCFDA and ROS-mediated oxidation of the fluorochrome was assayed by flow cytometry. The results show that treatment with GO-201, and not CP-1, increases intracellular ROS levels (Fig. 2C). Similar results were obtained with MCF-7 cells (Supplementary Fig. S1B). Increases in ROS above the reducing capacity of the cell can result in the formation of DNA double-strand breaks. The recognition of such DNA damage is associated with the activation of the ataxia telangiectasia–mutated (ATM) and Rad-3–related kinases, which in turn phosphorylate the histone variant H2AX and the checkpoint-1 kinase (Chk1; ref. 32). In concert with this model and the observed increases in ROS, treatment of ZR-75-1 breast cancer cells with GO-201 was associated with activation of ATM and phosphorylation of H2AX and Chk1 (Fig. 2D). Similar results were obtained when MCF-7 cells were treated with GO-201 (Supplementary Fig. S1C). These findings indicate that GO-201 disrupts MUC1 function and redox balance and, in turn, activates the DNA damage response.

**GO-201 induces S phase arrest and death.** To determine whether exposure to GO-201 affects growth, ZR-75-1 cells were treated with 5 μmol/L GO-201 for 3 days and monitored for cell cycle distribution. Significantly and consistent with activation of the DNA damage response, there was a substantial arrest in the S phase compared with that in cells left untreated or treated with CP-1 (Fig. 3A). By day 4, the S-phase population was decreased, potentially through attrition with cell death (Fig. 3A). There was little, if any, accumulation of cells with a distinct sub-G1 DNA peak to support the induction of apoptosis (Fig. 3A). Moreover, there was no detectable activation of caspase-3 (data not shown). However, oxidative stress is also associated with nonapoptotic forms of death, which include necrosis (33). In this regard, treatment of ZR-75-1 cells with GO-201, and not CP-1, was associated with the appearance of cells with DNA that had undergone extensive degradation consistent with necrosis (Fig. 3B). In addition, treatment with GO-201 was associated with uptake of propidium iodide as a measure of loss of membrane integrity, which was detectable by day 3 and more prominent by day 4 (Fig. 3B). The MCF-7 cells responded similarly to GO-201 with the arrest of growth in the S phase and the absence of cells with a distinct sub-G1 peak (Fig. 3C). Treatment of MCF-7 cells with GO-201 was also associated with loss of membrane integrity (Fig. 3D). These findings indicate that GO-201 inhibits growth and induces necrosis of human breast cancer cells. However, given potential difficulties in distinguishing late apoptosis from necrosis (30), the results do not exclude the possibility that some of the cells may have died by a late apoptotic response.

**GO-201 targets MUC1-expressing breast carcinoma cells.** Human MDA-MB-231 breast cancer cells express endogenous MUC1 (7), but unlike ZR-75-1 and MCF-7 cells, these cells are negative for ER, PR, and ErbB2 (triple negative; ref. 34). Notably, as found for ZR-75-1 and MCF-7 cells, GO-201, and not CP-1, treatment was associated with the arrest of MDA-MB-231 cell growth (Fig. 4A), accumulation of cells in S phase with the absence of a distinct sub-G1 peak (data not shown), and the loss of cell membrane integrity (Supplementary Fig. S2). Moreover, GO-201 treatment of MUC1-expressing primary breast cancer cells in short-term culture resulted in growth arrest (Fig. 4B) and death (Fig. 4C). In contrast to these results, GO-201 had no effect on growth of MUC1-negative 293 kidney epithelial cells (Supplementary Fig. S3). Studies were also performed on the MCF-10A nontransformed mammary epithelial cell line (35, 36), which
expresses MUC1, but at levels lower than that found in ZR-75-1 and MCF-7 cells (8). Unlike the breast cancer cells, GO-201 had no effect on MCF-10A cell growth (Fig. 4D), cell cycle distribution (Supplementary Fig. S4), or death (data not shown). These findings indicate that GO-201 targets breast carcinoma cells that are addicted to endogenous MUC1.

GO-201 inhibits tumorigenicity of estrogen-dependent and estrogen-independent breast cancers. To assess antitumor activity, ZR-75-1 cells were implanted s.c. into the flanks of nude mice. Mice bearing tumors of ~150 mm³ were treated with GO-201 at doses of 10 and 50 mg/kg/d. Administration of GO-201 at 10 mg/kg/d for 21 days slowed growth compared with that obtained with vehicle (PBS) alone or with CP-1 given at 50 mg/kg/d for 21 days (Fig. 5A). In addition, administration of GO-201 at 50 mg/kg/d had no effect on body weight (Supplementary Fig. S5) and blocked tumor growth over the initial 6 days of treatment (Fig. 5A). Consequently, treatment was stopped, and there was no detectable growth of the tumors over the next 17 days (Fig. 5A). To assess in part the basis for the activity, tumors harvested on day 24 from control and GO-201–treated (50 mg/kg/d) mice were examined by histopathology. Tumors from the treated mice were markedly necrotic compared with those from mice treated with the vehicle or CP-1 (Fig. 5B). In other studies with somewhat larger tumors (~275 mm³), administration of GO-201 at an intermediate dose of 30 mg/kg/d × 21 days was also associated with the arrest of tumor growth (Fig. 5C). Moreover, these tumors exhibited areas with necrosis, cellular debris, and swollen cells with cytoplasmic vacuoles (Fig. 5D).

To extend these findings, additional groups of 10 mice bearing ZR-75-1 tumors were treated with GO-201 at 30 mg/kg/d × 21 days and followed for longer periods (Fig. 6A). The GO-201 dose of 30 mg/kg/d was selected based on the findings that 10 mg/kg/d partially inhibits tumor growth compared with complete growth inhibition at both 30 and 50 mg/kg/d. In addition and to determine whether this activity is dependent on all 15 MUC1 amino acids in GO-201, we synthesized GO-202, a shorter CQCRRK peptide with the poly-d-Arg transduction domain (Supplementary Fig. S6). As found...
with GO-201, treatment of ZR-75-1 cells with GO-202 in vitro was associated with growth arrest and induction of necrosis (Supplementary Fig. S6; data not shown). Moreover, like GO-201, treatment of ZR-75-1 tumors with GO-202 at 30 mg/kg/d × 21 days resulted in the arrest of growth (Fig. 6A). Significantly, tumors treated with GO-201 or GO-202 were no longer palpable by days 49 to 56 (Fig. 6A). On day 63, one mouse from each treated group was sacrificed to assess the s.c. region implanted with ZR-75-1 tumor cells. There was no visual evidence for the remaining tumor at the implantation site or spread to other organs. Histopathologic examination of the implantation site further supported the absence of tumor cells (Fig. 6B). The remaining nine mice in each treatment group are being followed for the reemergence of tumors. As of day 152, none of the nine remaining mice in each treated group had evidence for tumor regrowth. To further assess antitumor activity, mice bearing MCF-7 xenografts were similarly treated with GO-201 and GO-202. As found for ZR-75-1 tumors, growth of the MCF-7 xenografts was arrested by both GO-201 and GO-202 treatment (Fig. 6C). By day 49, none of the treated mice had palpable tumors (Fig. 6C). One mouse from each treatment group sacrificed on day 49 had no visual evidence of the

**Figure 2.** GO-201 blocks MUC1-C function. **A,** ZR-75-1 cells were incubated with 5 μmol/L FITC-labeled GO-201 for the indicated times and then analyzed by flow cytometry. MFI is included in each of the panels. **B,** ZR-75-1 cells were untreated and treated with 5 μmol/L GO-201 or CP-1 each day for 3 d. Whole-cell lysates (WCL; left) and nuclear lysates (right) were immunoblotted with the indicated antibodies. **C,** ZR-75-1 cells were untreated (Control) and treated with 5 μmol/L GO-201 or CP-1 for 36 h. The cells were then incubated with c-H2DCFDA for 30 min, and fluorescence of oxidized c-H2DCF was measured by flow cytometry. **D,** ZR-75-1 cells were treated with 5 μmol/L GO-201 each day for 3 d. Lysates were subjected to immunoblotting with the indicated antibodies against ATM, H2AX, and Chk1.
remaining tumor at the implantation site or spread to other organs. There was also no evidence for remaining tumor cells at the implantation site by histopathologic analysis (Supplementary Fig. S7A). Moreover, none of the remaining mice have had recurrence of tumors as of day 136. To determine whether targeting MUC1 also inhibits tumorigenicity of estrogen-independent breast cancer cells, we treated MDA-MB-231 tumor xenografts with GO-201 or GO-202. As found for ZR-75-1 and MCF-7 tumors, growth of the MDA-MB-231 xenografts was arrested by GO-201 and GO-202 treatment (Fig. 6D). Tumors in the treated mice were no longer palpable by day 42, and on day 49, animals were sacrificed to assess the remaining tumor. Again, there was no visual evidence for tumor at the implantation site or in other organs. Moreover, histopathology confirmed the absence of tumor cells at the implantation site (Supplementary Fig. S7B). None of the nine remaining mice in each treated group had evidence for tumor progression on day 138 and are being followed for recurrence. These findings with MDA-MB-231 and the estrogen-dependent ZR-75-1 and MCF-7 tumors indicate that targeting MUC1 with GO-201 or GO-202 is associated with regression and prolonged arrest of tumor regrowth.

Discussion

GO-201 blocks MUC1 oligomerization. MUC1-induced transformation is abrogated by mutation of the CQC sequence in the cytoplasmic domain to AQA, indicating that this motif is of
importance to the transforming function (18). MUC1 forms oligomers, and the CQC motif is necessary for this oligomerization (18). In addition, oligomer formation is necessary for targeting of the MUC1-C subunit to the nucleus (18) and function of MUC1-C in maintaining redox balance in carcinoma cells (27–29). Based on these findings, we reasoned that disruption of MUC1 oligomerization would have the potential to block the MUC1 transforming function. The present studies show that GO-201 inhibits oligomerization of MUC1-CD in vitro. MUC1-CD forms dimers with a Kd of 33 nmol/L (18). GO-201 similarly bound to MUC1-CD with a Kd of 33 nmol/L (18). GO-201 inhibited oligomerization of MUC1-CD in vitro. MUC1-CD forms dimers with a Kd of 33 nmol/L (18). GO-201 similarly bound to MUC1-CD with a Kd of 33 nmol/L (18).
30 nmol/L. In addition, the demonstration that the mutated control CP-1 peptide has little, if any, effect on MUC1 oligomerization provided support for the dependence on CQC motif. GO-201, and not CP-1, was also effective in blocking MUC1-C oligomerization and suppression of ROS in cells. While completing these studies, a peptide (PMIP) corresponding to another region of MUC1-CD was shown to inhibit binding of MUC1 to β-catenin and EGFR (37). In contrast to GO-201, which interacts directly with MUC1, PMIP acts as a decoy to interact with MUC1 binding partners. Thus, GO-201 and PMIP represent different strategies to inhibit MUC1 function.

**Selectivity of GO-201 for MUC1 overexpressing carcinoma cells.** Consistent with nuclear targeting of MUC1 being dependent on oligomerization (18), uptake of GO-201 in ZR-75-1 cells was associated with down-regulation of MUC1-C levels in the nucleus. Similar results were obtained with MCF-7 breast cancer cells, indicating that this response to GO-201 is not cell specific. Moreover, exposure of these cells to GO-201, and not CP-1, was associated with ROS-induced activation of the DNA damage response, S-phase growth arrest, and induction of late apoptosis/necrosis. These effects of directly inhibiting MUC1 with GO-201 are in contrast to that obtained with the PIMP decoy peptide, which reduces proliferation without evidence for inducing cell death (37). Of importance is whether GO-201 induces death by a mechanism dependent on the expression of its intended target or if it functions as a nonspecific cytotoxin. In that context, GO-201 had no effect on

![Figure 5](https://example.com/figure5.png)
MUC1-negative 293 cells. In addition, exposure of nonmalignant MCF-10A mammary epithelial cells to GO-201 had no apparent effect. These findings indicate that sensitivity to GO-201 is dependent on a function of MUC1, such as suppression of ROS, associated with the malignant phenotype. GO-201, thus, seems to have an activity that is selective for carcinoma cells that overexpress MUC1.

Antitumor activity associated with disrupting MUC1 function. An overriding question was whether GO-201 could be delivered in vivo with an effective therapeutic index, that is antitumor activity and an acceptable toxicity profile. In addressing this issue, we found that administration of GO-201 was well-tolerated without apparent acute toxicities. We also found that GO-201 treatment is effective in inducing tumor regression and prolonged delays in regrowth. These results were in contrast to the administration of the control CP-1, which had no antitumor activity. These results are explained, at least in part, by the finding that treatment with GO-201 is associated with the induction of tumor necrosis. Similar effects were observed with the ZR-75-1, MCF-7, and MDA-MB-231 tumor models, indicating that GO-201 is active against estrogen-dependent and triple-negative breast cancer cells; the latter being insensitive to currently available targeted therapies (38). The effects of directly inhibiting MUC1 with GO-201 are in contrast to that obtained with the PIMP decoy peptide that inhibits growth of MDA-MB-231 tumors without inducing complete regressions (37). Importantly, in this regard, the ZR-75-1, MCF-7, and MDA-MB-231 tumors treated with GO-201 have had no evidence for recurrence as of days 136 to 152.

Targeting of MUC1 function. Overexpression of MUC1, as found in human breast tumors, blocks death in the response to oxidative stress, DNA damage, and hypoxia (14, 20, 26–29). Overexpression of MUC1 also induces anchorage-independent growth

Figure 6. GO-201 and GO-202 induce regression of ZR-75-1, MCF-7, and MDA-MB-231 tumors. A–C, female BALB/c nu/nu mice were implanted with 17β-estradiol plugs. After 24 h, ZR-75-1 (A) or MCF-7 (C) breast cancer cells (1 × 10^7) derived from xenografts were injected s.c. in the flank. When ZR-75-1 and MCF-7 tumors were 140 to 170 mm^3 and 175 to 225 mm^3, respectively, the mice were pair matched into groups of 10 mice and injected i.p. with PBS (closed squares), 30 mg/kg GO-201 each day for 21 d (closed triangles), or 30 mg/kg GO-202 each day for 21 d (open circles). Mice were weighed twice weekly, and tumor measurements were performed every 2 d. After 5 wk, the tumors were measured once each week. There was no evidence of weight loss in the treated groups. Points, mean tumor volumes; bars, SE (<10%). For H&E staining, (a) control ZR-75-1 and MCF-7 tumors were harvested on day 14, (b) the ZR-75-1 cell implantation sites from treated mice were obtained on day 63 (B), and (c) the MCF-7 cell implantation sites from treated mice were obtained on day 49 (Supplementary Fig. S7 A). D, 4- to 6-wk-old female BALB/c nu/nu mice were injected s.c. in the flank with MDA-MB-231 cells (1 × 10^7) derived from xenografts. When tumors were 240 to 280 mm^3, the mice were pair matched into groups of 10 mice and injected i.p. with PBS (closed squares), 30 mg/kg GO-201 each day for 21 d (closed triangles), or 30 mg/kg GO-202 each day for 21 d (open circles). Control tumors harvested on day 14, and the MDA-MB-231 cell implantation sites from the treated mice obtained on day 49, were stained with H&E (Supplementary Fig. S7 B).
and tumorigenicity (12, 24). However, there has been no evidence that targeting MUC1 oligomerization can affect survival or tumorigenicity of human breast cancer cells. The present results with GO-201 emphasize the importance of MUC1 in contributing to breast cancer cell survival and tumorigenicity. To provide further support for the observed activity with GO-201, we synthesized GO-202, which also targets the CQC motif. We found that GO-202 behaves like GO-201 in vitro and in the xenograft models, indicating that the CQC motif is indeed an Achilles heel of the MUC1 oncoprotein. The present results also suggest that the breast cancer cells studied here are addicted to MUC1 function. In this context, oncogene addiction has been defined as dependence of a cancer cell on a single gene product for maintenance of the malignant phenotype (39). Notably, therapies that target addiction to specific oncoproteins have been associated with the emergence of (a) circumventing mutations and/or (b) dependence on other transforming genes. Thus, it is conceivable that targeting the MUC1 CQC motif could result in resistant mutants. Alternatively, targeting MUC1 could select for the emergence of breast cancer cells that are dependent on other oncoproteins. Whereas these issues will need to be addressed in subsequent studies, the loss of tumorigenicity in the xenograft models suggests that targeting MUC1 by blocking oligomerization can have prolonged effects.

Disclosure of Potential Conflicts of Interest

D. Kufe is a founder of Genus Oncology and a consultant to the company. D. Raina, L. Yin, and S. Kharbanda are employees of Genus Oncology. The other authors disclosed no potential conflicts of interest.

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