Dependence on the MUC1-C Oncoprotein in Non–Small Cell Lung Cancer Cells

Deepak Raina, Michio Kosugi, Rehan Ahmad, Govind Panchamoorthy, Hasan Rajabi, Maroof Alam, Takeshi Shimamura, Geoffrey I. Shapiro, Jeffrey Supko, Surender Kharbanda, and Donald Kufe

Abstract

Non–small cell lung cancer (NSCLC) cells are often associated with constitutive activation of the phosphoinositide 3-kinase (PI3K) → Akt → mTOR pathway. The mucin 1 (MUC1) heterodimeric glycoprotein is aberrantly overexpressed in NSCLC cells and induces gene signatures that are associated with poor survival of NSCLC patients. The present results show that the MUC1 C-terminal subunit (MUC1-C) cytoplasmic domain associates with PI3K p85 in NSCLC cells. We show that inhibition of MUC1-C with cell-penetrating peptides blocks this interaction with PI3K p85 and suppresses constitutive phosphorylation of Akt and its downstream effector, mTOR. In concert with these results, treatment of NSCLC cells with the MUC1-C peptide inhibitor GO-203 was associated with downregulation of PI3K → Akt signaling and inhibition of growth. GO-203 treatment was also associated with increases in reactive oxygen species (ROS) and induction of necrosis by a ROS-dependent mechanism. Moreover, GO-203 treatment of H1975 (EGFR L858R/T790M) and A549 (K-Ras G12S) xenografts growing in nude mice resulted in tumor regressions. These findings indicate that NSCLC cells are dependent on MUC1-C both for activation of the PI3K → Akt pathway and for survival. Mol Cancer Ther; 10(5); 806–16. © 2011 AACR.
Targeting MUC1-C in NSCLC

receptor (15). The MUC1-C extracellular domain interacts with the ligand galectin-3 and thereby forms complexes with EGFR (16). The available evidence indicates that MUC1-C promotes EGFR-mediated signaling (17–19). In this context, the MUC1-C cytoplasmic domain functions as a substrate for EGFR and c-Src phosphorylation (17, 20). In turn, the MUC1-C pYEEKV motif serves as a binding site for the c-Src SH2 domain (20). The MUC1-C cytoplasmic domain also contains a YTNP site that, when tyrosine phosphorylated, interacts directly with the SH2 domain of the Grb2 adapter protein (21, 22). The MUC1-C/Grb2 complex associates with the Ras activator SOS (son-of-sevenless), linking MUC1-C to the Ras pathway (21). Importantly, MUC1-C activates the PI3K → Akt pathway (23) and the MUC1-C cytoplasmic domain has a YHPM site that following phosphorylation functions as a binding site for the PI3K SH2 domain (24). Overexpression of MUC1 as found in human carcinomas is associated with accumulation of MUC1-C in the cytoplasm and targeting of MUC1-C to the nucleus (15). The overexpression of MUC1-C has also been directly associated with activation of β-catenin (25), IKK → NF-kB RelA (26, 27), and STAT1/3 (28, 29) signaling. In concert with these functions, an inhibitor of MUC1-C oligomerization blocks MUC1-C–mediated activation of the NF-kB and STAT pathways (27–29). In addition, treatment of human breast and prostate tumor xenografts in nude mice with the MUC1-C inhibitors GO-201 and GO-202 is associated with complete and prolonged regressions (30, 31). Recent work has shown that MUC1-C induces gene signatures that are highly predictive of overall and disease-free survival of NSCLC patients (32, 33). Importantly, silencing of MUC1 expression in NSCLC cells is associated with downregulation of STAT3 activation and loss of survival (34).

The present studies show that MUC1-C associates with PI3K in NSCLC cells through direct binding of the MUC1-C cytoplasmic domain and the PI3K p85 SH2 domain. The results show that inhibition of MUC1-C function blocks activation of the PI3K → p-Akt → p-mTOR pathway. The results also show that the MUC1 inhibitor GO-203 increases reactive oxygen species (ROS) and induces necrotic death of NSCLC cells in vitro. Moreover, we show that GO-203 is effective in inducing regressions of NSCLC tumor xenografts in mice.

Materials and Methods

Cell culture

Human H1975 (35), H1650 [American Type Culture Collection (ATCC); ref 36], HCC827 (35), A549, H2228 (13), H460 (37), H1299 (38), and NCI-H292 (39) NSCLC cells were all obtained from the ATCC and passaged for less than 6 months and then replaced with early passage frozen stocks. No further authentication was carried out. The cells were grown in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L l-glutamine. Normal lung epithelial cells (NLEC; Lonza) were cultured in epithelial cell growth medium. Cells were treated with GO-201, GO-202, GO-203, CP-1, and CP-2 peptides (AnaSpec, Inc.; all dissolved in PBS), N-acetylcysteine (NAC; Sigma) and Tiron (Sigma). Viability was determined by trypan blue exclusion. Cells were transiently transfected with siRNA pools (Dharmacon) in the presence of Lipofectamine 2000 (Invitrogen).

Immunoprecipitation and immunoblot analysis

Cell lysates were prepared as described (30). Soluble proteins were immunoprecipitated with anti-MUC1-C (Ab5; Neomarkers), anti-EGFR (Abcam), or a control IgG. The precipitates and lysates not subjected to immunoprecipitation were immunoblotted with anti-MUC1-C, anti-β-actin (Sigma-Aldrich), anti-p-EGFR (Cell Signaling Technology), anti-EGFR, anti-p-Akt, anti-Akt, anti-p-mTOR, anti-mTOR (Cell Signaling Technology), anti-P3K p85 (Millipore), and anti-p-Tyr (4G10; Millipore). Reactivity was detected with horseradish peroxidase–conjugated secondary antibodies and chemiluminescence.

Quantitative real-time PCR

Total RNA was isolated from cells using the RNeasy Mini Kit (Qiagen). For quantitative real-time PCR (qRT-PCR), cDNA was synthesized with 1 μg of total RNA, using the Superscript-III First-Strand Synthesis SuperMix (Invitrogen). The SYBR Green qPCR Assay Kit (Applied Biosystems) was used with 1 μL of cDNA for amplification with the ABI Prism 7000 Sequence Detector (Applied Biosystems). Primers used for qRT-PCR are listed in the Supplementary Table. Relative enrichment was calculated as described (28), and the results are expressed as the mean ± SD of triplicate values for each sample.

In vitro binding assays

Glutathione-S-transferase (GST) and GST-P3K p85 SH2 (Pierce Biotechnology) were bound to glutathione beads and incubated with cell lysates as described (26). Alternatively, purified His-MUC1-CD was incubated with recombinant c-Met (Upstate Cell Signaling Solutions) in kinase buffer containing 15 μmol/L ATP for 15 minutes at room temperature. Samples were washed and then incubated with GST or GST-P3K p85 SH2. Adsorbates were analyzed by immunoblotting.

Cell transfections

HEK293 cells were transfected with pIRES-puro2-MUC1 or pIRES-puro2-MUC1(Y20F) in the presence of Lipofectamine 2000 as described (40). The cells were harvested at 48 hours after transfection for coimmunoprecipitation studies.

Analysis of cell-cycle distribution and cell membrane integrity

Cells were fixed with 80% ethanol and incubated in PBS containing RNase and propidium iodide as
described (30). 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 minutes at room temperature and then monitored by flow cytometry as described (30).

**Measurement of ROS levels**

Cells were incubated with 10 μmol/L c-H2DCFDA (Molecular Probes) for 20 minutes at 37°C. Fluorescence of oxidized c-H2DCF was measured as described (31).

**NSCLC tumor xenograft models**

Four- to 6-week-old BALB/c nu/nu male/female mice were injected subcutaneously with 1 × 10⁷ H1975 or A549 cells in the flank. When tumors were detectable, the mice were pair-matched into control and treatment groups of 10 mice each. Mice were excluded if the tumors were not within 15% of the mean volume. PBS (control vehicle), 30 mg/kg body weight GO-203, or 30 mg/kg body weight CP-2 (peptides dissolved in PBS) were administered daily by intraperitoneal injection for 21 days. Tumor volume (V) was calculated using the formula V = L² × W/2, where L and W are the larger and smaller diameters, respectively. Tumors and sites of tumor implantation were evaluated by staining with hematoxyline and eosin (H&E).

**Statistical analysis**

The analysis of data was conducted using the 2-tailed, unpaired Student’s t test. P values greater than 0.05 were considered significant.

**Results**

**Effects of inhibiting MUC1-C with GO-201 on growth and survival of NSCLC cells**

Previous work has shown that MUC1-C is expressed in NSCLC cell lines (34). In concert with those findings, immunoblot analysis of H1975 (EGFR L858R/T790M), H1650 (EGFR delE746-A750), and HCC827 (EGFR delE746-A750) NSCLC cells confirmed expression of the MUC1-C subunit (Fig. 1A). To assess sensitivity to inhibition of MUC1 function, H1975 cells were treated with the MUC1-C peptide inhibitors GO-201 ([R]⁹-CQCRRKNYGQLDIFP) and GO-202 ([R]⁹-CQCRRKN). Treatment of H1975 cells with 5 μmol/L GO-201 or GO-202 was associated with an initial inhibition of growth and then a decrease in cell number (Fig. 1B).
contrast, CP-1 had little if any effect (Fig. 1B). H1650 and HCC827 cells also responded to GO-201, and not CP-1, with suppression of growth (Fig. 1C). Similar results were obtained with HCC827 cells (Fig. 1D). These findings indicated that MUC1-C is a potential target for certain NSCLC cells with activating EGFR mutations.

**GO-203 inhibits growth and survival of NSCLC cells**

Human A549 NSCLC cells harbor a K-Ras G12S mutation and express MUC1-C at elevated levels compared with that in NLECs (Fig. 2A). Growth of A549 cells was substantially inhibited by GO-201 and the shorter version, GO-202 (Fig. 2B). Conversion of GO-202 to the D-amino acid configuration, designated GO-203, was then tested to determine whether such modification affects activity. In this regard, like GO-201 (30), GO-203 blocked oligomerization of the MUC1-C cytoplasmic domain (Supplemental Fig. S1). Moreover, GO-203 was effective in inhibiting A549 cell growth (Fig. 2C, left). In a control peptide, designated CP-2, alteration of the CQC motif to AQA abrogated the growth inhibitory effects (Fig. 2C, left). In contrast to the effects on A549 cells, GO-203 had no significant effect on NLEC growth (Fig. 2C, right). Human H2228 NSCLC cells have the EML4-ALK translocation and express MUC1-C at somewhat higher levels than that in H1650 cells (Fig. 2D, left). As found for A549 cells, H2228 cells were also sensitive to GO-202 and GO-203 (Fig. 2D, right), further indicating that the D-amino acid configuration is as active as the L-form. Indeed,

![Figure 2. Growth of NSCLC cells with K-Ras and EML4-ALK mutations is inhibited by blocking MUC1-C function.](image-url)
growth of H1975, H1650, and HCC827 cells was similarly inhibited by GO-203 (data not shown).

To further assess sensitivity of NSCLC cells to GO-203, we studied NCI-H292 (wild-type EGFR, wild-type K-Ras), H1299 (wild-type EGFR, wild-type K-Ras), and H460 (wild-type EGFR, K-Ras G61H) NSCLC cells. MUC1 mRNA levels were higher in NCI-H292 cells than in A549 cells and were similar in H1299 cells (Supplementary Fig. S2A). In contrast, H460 cells had a lower abundance of MUC1 mRNA (Supplementary Fig. S2A). Immunoblot analysis confirmed a relatively higher level of MUC1-C protein in NCI-H292 cells than that in H1299 and H460 cells (Supplementary Fig. S2B). NCI-H292 and H1299 cells responded to GO-203 treatment with an initial slowing of growth and then a decline in cell number over days 4 to 8 (Supplementary Fig. S2C and D). Growth of H460 cells, which express relatively lower levels of MUC1-C, was also slowed; however, there was no significant decrease in cell number after day 4 (Supplementary Fig. S2E). These findings indicate that (i) sensitivity of NSCLC cells to GO-203 is independent of the EGFR or K-Ras mutational status and (ii) a level of MUC1-C expression below a certain threshold, as observed in H460 cells, may confer less dependence on MUC1-C for survival.

MUC1 levels have been shown to be independent of EGFR expression in NSCLC cells (41). To assess the effects of EGFR on MUC1-C, we downregulated EGFR with siRNA pools in H1975 cells (Supplementary Fig. S3A). Silencing EGFR had no detectable effect on MUC1-C abundance (Supplementary Fig. S3A). Silencing of K-Ras in A549 cells also had no effect on MUC1-C levels (Supplementary Fig. S3B), indicating that MUC1-C expression is independent of EGFR and K-Ras signaling in these cells.

Figure 3. GO-203 blocks binding of the MUC1-C cytoplasmic domain to the PI3K p85 SH2 domain. H1975 (A) and A549 (B) cells were left untreated (control) and treated with 5 μmol/L GO-203 or CP-2 each day for 2 days. Lysates were precipitated with anti-MUC1-C or a control IgG. The precipitates were immunoblotted with anti-PI3K p85 and anti-MUC1-C. C, amino acid sequence of the MUC1-C cytoplasmic domain (MUC1-CD). The indicated phosphorylation sites are highlighted, and the YHPM is boxed. His-MUC1-CD was incubated with GST or GST-Pi3K SH2 in the presence and absence of c-Met and ATP. GST or GST-Pi3K p85 SH2 bound to glutathione beads was then added to the reaction products (left). c-Met-phosphorylated His-MUC1-CD was incubated with GST-Pi3K SH2 in the absence and presence of increasing amounts of GO-203 (right). The adsorbates and inputs were immunoblotted with anti-MUC1-C, D, His-MUC1-CD and His-MUC1-CD(Y20F) were incubated with c-Met and ATP. GST or GST-Pi3K p85 SH2 bound to glutathione beads was then added to the reaction products (left). c-Met-phosphorylated His-MUC1-CD was incubated with GST-Pi3K SH2 in the presence and absence of GO-203 (right). The adsorbates and inputs were immunoblotted with anti-MUC1-C. C, amino acid sequence of the MUC1-C cytoplasmic domain (MUC1-CD). The indicated phosphorylation sites are highlighted, and the YHPM is boxed. His-MUC1-CD was incubated with GST or GST-Pi3K SH2 in the presence and absence of c-Met and ATP. GST or GST-Pi3K p85 SH2 bound to glutathione beads was then added to the reaction products (left). c-Met-phosphorylated His-MUC1-CD was incubated with GST-Pi3K SH2 in the absence and presence of increasing amounts of GO-203 (right). The adsorbates and inputs were immunoblotted with anti-MUC1-C.
GO-203 disrupts the interaction between MUC1-C and PI3K p85

The MUC1-C cytoplasmic domain associates with PI3K p85 in transfected HEK293 cells (24). To determine whether endogenous MUC1-C associates with PI3K in NSCLC cells, coprecipitation studies were conducted using H1975 cell lysates. The results showed that MUC1-C associates with PI3K p85 (Fig. 3A). Moreover, treatment of the H1975 cells with GO-203, but not CP-2, blocked the interaction between MUC1-C and PI3K p85 (Fig. 3A). Similar results were obtained in coprecipitation experiments using A549 cell lysates (Fig. 3B). The MUC1-C cytoplasmic domain contains a YHPM motif that, when phosphorylated, functions as a binding motif for the PI3K p85 SH2 domains (Fig. 3C). To determine whether GO-203 blocks the direct interaction, we first incubated the MUC1-C cytoplasmic domain with the PI3K p85 SH2 domain. As expected, there was no detectable binding (Fig. 3C, left). However, in concert with previous studies showing that the YHPM site is phosphorylated by c-Met (15), recombinant c-Met-mediated phosphorylation of the YHPM site was associated with binding to the PI3K p85 SH2 domain (Fig. 3C, left). In addition, the interaction between the phosphorylated MUC1-C cytoplasmic domain and the PI3K p85 SH2 domain was attenuated by including increasing amounts of GO-203 to the reactions (Fig. 3C, right). As further evidence for the involvement of the pYHPM motif, mutation of the MUC1-C cytoplasmic domain at Y-20 to F (Y20F) blocked the interaction with the PI3K p85 SH2 domain (Fig. 3D, left). Transfection of MUC1-null HEK293 cells with a vector expressing MUC1 or MUC1(Y20F) also showed that coprecipitation of MUC1-C with PI3K p85 is abrogated by the Y20F mutation (Fig. 3D, right). These findings indicated that MUC1-C associates with PI3K through direct binding to the pYHPM motif in the MUC1-C cytoplasmic domain and that GO-203 blocks the interaction.

Figure 4. Inhibition of MUC1-C downregulates the PI3K -- Akt -- mTOR pathway. A, H1975 cells were left untreated (control) and treated with 5 μmol/L GO-203 or CP-2 each day for 2 days. Lysates were immunoblotted with the indicated antibodies. B, H1650 (left) and HCC827 (right) cells were left untreated and treated with 5 μmol/L GO-203 or CP-2 each day for 2 days. Lysates were immunoblotted with the indicated antibodies. C, A549 cells were left untreated and treated with 5 μmol/L GO-203 or CP-2 each day for 2 days. Lysates were immunoblotted with the indicated antibodies. D, H2228 cells were left untreated and treated with 5 μmol/L GO-203 or CP-2 each day for 2 days. Lysates were immunoblotted with the indicated antibodies.
Treatment of H1975 or A549 cells with GO-203 had no effect on the interaction between EGFR and PI3K p85, indicating that the inhibitory effects of GO-203 on the PI3K → Akt → mTOR pathway are not attributable to disruption of the interaction between PI3K p85 and EGFR (Supplementary Fig. S4A and B). Moreover, GO-203 treatment of H1975 cells was associated with a modest decrease in phosphorylation of MUC1-C on tyrosine (Supplementary Fig. S4C) and no apparent effect on MUC1-C tyrosine phosphorylation in A549 cells (Supplementary Fig. S4D).

**Inhibition of MUC1-C downregulates the PI3K → Akt pathway**

On the basis of the aforementioned observations, we asked whether disruption of the interaction between MUC1-C and PI3K p85 with GO-203 affects phosphorylation of Akt. Significantly, treatment of H1975 cells with GO-203, and not CP-2, was associated with inhibition of Akt phosphorylation, consistent with downregulation of PI3K signaling (Fig. 4A). Treatment with GO-203 was also associated with inhibition of the PI3K-related mTOR pathway (Fig. 4A). Moreover, GO-203 was effective in downregulating p-Akt and p-mTOR in H1650 and HCC827 cells (Fig. 4B), indicating that inhibition of MUC1-C suppresses PI3K activity in these NSCLC cells with EGFR mutations. In A549 cells, GO-203 treatment was similarly associated with suppression of p-Akt and p-mTOR levels, providing further support for downregulation of PI3K activity (Fig. 4C, left). In addition and as found in H1975 cells, GO-203–induced downregulation of p-Akt and p-mTOR in A549 cells was detectable at 24 and 48 hours (Fig. 4C, right), preceding the onset of cell death. Consistent with effects that extend beyond NSCLC cells with EGFR or K-Ras mutations, H2228 cells with the EML4-ALK translocation responded to GO-203, and not CP-2, with decreases in Akt and mTOR phosphorylation (Fig. 4D). These findings indicate that inhibition of MUC1-C in diverse types of NSCLC cells blocks the interaction between MUC1-C and PI3K p85 and suppresses activation of the PI3K → Akt → mTOR pathway.

**GO-203 induces necrotic cell death by a ROS-mediated mechanism**

Inhibition of the PI3K signaling pathway in NSCLC cells expressing mutant EGFR or K-Ras leads to growth arrest and not cell death (42–44). In this regard, the aforementioned studies showed that inhibition of MUC1-C in H1975 and A549 cells is associated with an initial slowing of growth and then a subsequent decrease in cell number consistent with cell death. Indeed, treatment of H1975 and A549 cells with MUC1-C inhibitors for 6 days was associated with (i) extensive DNA degradation, (ii) no identifiable sub-G1 peak, and (iii) loss of cell membrane integrity (Supplementary Fig. S5A and B; data not shown), consistent with the induction of necrotic cell death. Other work has shown that inhibition of MUC1-C increases ROS (30, 31); however, it is not known whether disruption of redox balance in this setting contributes to

---

**Figure 5.** GO-203 induces death by a ROS-mediated mechanism. **A,** H1975 cells were left untreated (control) and treated with 5 μmol/L GO-203 in the absence or presence of 1 mmol/L NAC for 3 days. The cells were then incubated with c-H2DCFDA for 30 minutes, and fluorescence of oxidized c-H2DCF was measured by flow cytometry. **B,** H1975 cells were left untreated (control) and treated with 5 μmol/L GO-203 in the absence or presence of 1 mmol/L NAC for 5 days. Cells were stained with propidium iodide and analyzed by flow cytometry. The percentage of necrotic cells is included. **C,** H1975 cells were left untreated (control) and treated with 5 μmol/L GO-203 in the absence or presence of 5 mmol/L Tiron for 3 days. The cells were then incubated with c-H2DCFDA for 30 minutes, and fluorescence of oxidized c-H2DCF was measured by flow cytometry. **D,** H1975 cells were left untreated (control) and treated with 5 μmol/L GO-203 in the absence or presence of 5 mmol/L Tiron for 5 days. Cells were stained with propidium iodide and analyzed by flow cytometry. The percentage of necrotic cells is included. PI, propidium iodide.
cell death. To address this possibility, H1975 cells were treated with GO-203 and the antioxidant NAC. Analysis of ROS levels showed that GO-203–induced increases in ROS are reversed by cotreatment with NAC (Fig. 5A). GO-203–induced necrosis of H1975 cells was also attenuated by NAC (Fig. 5B). To search for further evidence for the involvement of ROS, H1975 cells were treated with Tiron, a superoxide scavenger. As found with NAC, Tiron blocked the GO-203–induced increases in ROS levels (Fig. 5C) and necrotic cell death (Fig. 5D). These findings and similar results in A549 cells (data not shown) indicated that GO-203 induces necrosis, at least in part, by a ROS-mediated mechanism.

**GO-203 induces regressions of established NSCLC tumors**

To assess the antitumor effects of GO-203, H1975 cells were implanted in the flanks of nude mice. Mice bearing tumors of approximately 125 mm³ were intraperitoneally injected with GO-203 and, as a control, CP-2 at a dose of 30 mg/kg/d for 21 days. GO-203, but not CP-2, treatment was associated with an initial inhibition of H1975 tumor growth and then a progressive decline in volume (Fig. 6A). By day 28, the GO-203–treated tumors were no longer palpable (Fig. 6A). One mouse from the GO-203–treated group was sacrificed on day 32 to assess residual tumor. There was no gross evidence for remaining tumor at the implantation site or metastases to the liver, lungs, and other organs. Histologic examination of the implantation site following GO-203 treatment identified a small cluster of remaining tumor cells that appeared necrotic. Notably, there was no recurrence of tumor as of day 180 when the mice were sacrificed. Treatment of established A549 tumors (~75 mm³) with GO-203 for 21 days was similarly associated with an initial slowing of growth and then regression (Fig. 6C). Examination of the implantation site in one of the GO-203–treated mice on day 36 showed necrotic debris without intact tumor cells (Fig. 6D). In addition, there was no evidence for metastatic spread. As of day 180, the other GO-203–treated mice were without recurrence, indicating that both H1975 and A549 tumors respond to GO-203 with prolonged complete regressions.

**Discussion**

**MUC1-C cytoplasmic domain interacts with PI3K in NSCLC cells**

Transformation induced by the MUC1-C subunit cytoplasmic domain is associated with activation of
the PI3K → Akt pathway (23, 25). However, it was not known whether the MUC1-C subunit interacts with PI3K in NSCLC cells, which are dependent on PI3K → Akt signaling for survival. The present studies support a model in which MUC1-C activates PI3K in NSCLC cells through a direct interaction. The results show that (i) MUC1-C associates with PI3K p85 in NSCLC cells and (ii) in concert with previous work (24), the MUC1-C cytoplasmic domain pYHPM motif functions as a binding site for the PI3K p85 SH2 domain. Proteins containing the pYXXM sequence have a high affinity for the PI3K N-terminal and C-terminal SH2 domains. Specifically, ErbB3 contains 6 YXXM motifs that when phosphorylated on tyrosine function as binding sites for PI3K (4, 45). Other activated RTKs, the adaptor molecule Grb2-associated binding protein 1, and the polyoma middle T antigen, also contain pYXXM sites for PI3K binding (4, 45). The present results provide evidence that the MUC1-C cytoplasmic domain plays a similar role during interaction with PI3K in NSCLC cells. The MUC1-C transmembrane receptor is positioned at the apical cell membrane of polarized epithelial cells and is sequestered from RTKs at the basolateral membranes (46). However, with loss of polarity associated with transformation, MUC1-C forms complexes with the ErbB family members FGF3, PDGF, and c-Met, which, in turn, induce tyrosine phosphorylation of the MUC1-C cytoplasmic domain (15). Thus, direct binding of PI3K p85 to MUC1-C with a phosphorylated YHPM motif could confer the conformational change in p85 that activates the PI3K p110 catalytic subunit. The interaction between MUC1-C and RTKs is transient in the epithelial stress response with reversible loss of polarity (15). In contrast, in carcinoma cells with irreversible loss of polarity, the constitutive interaction between MUC1-C and RTKs could contribute to aberrant activation of the PI3K → Akt pathway that has been identified in diverse cancers.

Inhibition of MUC1-C downregulates the PI3K pathway

Dimerization of MUC1-C is dependent on a CQC motif in the cytoplasmic domain and is necessary for interactions between MUC1-C and certain effectors such as NF-κB RelA and STAT1/3 (27–29). GO-201 is a cell-penetrating peptide that contains the CQC sequence and binds directly to the MUC1-C cytoplasmic domain, thus blocking oligomerization (30). A second-generation configuration, GO-203, was synthesized with all d-amino acids that can afford greater stability in the presence of peptidases and, like GO-201 (30), was found to block dimerization of the MUC1-C cytoplasmic domain. Our results show that (i) GO-203 is as effective as GO-201 in inhibiting NSCLC cell growth and survival and (ii) treatment of NSCLC cells with GO-203 blocks the interaction between MUC1-C and PI3K p85. Similar results were obtained with GO-203 in in vitro binding studies of MUC1-C and the PI3K p85 SH2 domain, indicating that

MUC1-C oligomerization and the pYHPM motif are both needed to confer the interaction with PI3K p85. Alternatively, binding of GO-203 to the MUC1-C cytoplasmic domain could alter the conformation of the pYHPM site and thereby block binding to the PI3K p85 SH2 domain. There are a number of PI3K inhibitors that are under clinical development that directly target PI3K p110 activity (4). The evidence presented here indicates that GO-203 also blocks PI3K p110 activity; however, this effect would be indirect, that is, by abrogating the interaction between the MUC1-C cytoplasmic domain and PI3K p85. Inhibition of MUC1-C was effective in suppressing p-Akt in H1975 cells and thus occurred in a setting of resistance to EGFR inhibitors. GO-203–induced downregulation of p-Akt was also found in A549 cells that harbor a K-Ras mutant, against which there are presently no effective targeted therapies. Akt functions as an activator of mTORC1, and inhibitors are under development that block the activity of both PI3K and mTOR. In this regard, mTORC1 is also regulated by other signals; for example, the LKB1-AMPK pathway that links cell metabolism with growth control (47). Significantly, like inhibition of p-Akt, GO-203 treatment was associated with downregulation of p-mTOR in both H1975 and A549 cells. The inhibition of p-mTOR is explained, at least in part, by the effects of GO-203 on p-Akt; however, the present results do not exclude the possibility that blocking MUC1-C function may affect other signals that activate mTORC1.

GO-203 is effective in inducing death of NSCLC cells

The finding that inhibition of MUC1-C suppresses growth of NSCLC cells, without or with EGFR and K-Ras mutations, is in concert with downregulation of the PI3K → Akt pathway (1–3, 42–44). Indeed, our results indicate that the inhibitory effects of the MUC1-C peptides are dependent on MUC1-C expression and are not due to the specific gene mutation status. Nonetheless, the effects of GO-203 on PI3K signaling were not sufficient to explain the associated loss of survival in that downregulation of this pathway in NSCLC cells with EGFR or K-Ras mutations results in growth arrest and not cell death (42–44). Accordingly, the findings that MUC1 suppresses ROS levels and thereby blocks death (15) invoked the possibility that GO-203 induces death by a ROS-dependent mechanism. Indeed, as found previously with GO-201 (30, 31), treatment with GO-203 was associated with increases in ROS. By extension, the antioxidants NAC and Tiron attenuated GO-203–induced cell death, consistent with a ROS-mediated loss of survival. These findings would seem to support 2 effects of GO-203, one on PI3K signaling and the other on redox imbalance. Nonetheless, these results do not exclude the possibility that downregulation of the prosurvival PI3K → Akt pathway could contribute to increased sensitivity of these NSCLC cells to ROS–induced necrotic death. In this regard, Akt has been linked to the oxidative stress.
response and may exert its tumorigenic potential only after evading ROS-induced senescence (48, 49). Given these circumstances, the impact of GO-203 on NSCLC cell growth and survival in vitro provided the experimental basis for evaluating the antitumor activity of GO-203 against NSCLC xenografts growing in mice. Previous work has shown that murine lung carcinomas driven by a PI3K p110 mutant are highly sensitive to a dual PI3K-mTOR inhibitor (8). However, preclinical models suggest that PI3K or dual PI3K-mTOR inhibitors are likely to be less effective against lung cancers with EGFR mutations (4). In addition, mouse lung cancer driven by mutant K-Ras does not respond substantially to the dual PI3K-mTOR inhibitor (8). In contrast, our studies show that GO-203 is highly effective against H1975 cells with the EGFR (T790M) mutation, achieving complete responses that are maintained after completing 21 days of drug dosing. A549 K-Ras mutant tumors also responded to GO-203 with complete responses and lack of recurrence. These findings indicate that GO-203–induced inhibition of MUC1-C is effective against (i) NSCLC cells with wild-type EGFR and K-Ras in vitro and (ii) NSCLC cells with mutant EGFR or K-Ras growing in vitro and in mouse models. Therefore, an agent, such as GO-203, that targets MUC1-C and has entered phase I clinical evaluation may be effective in the treatment of NSCLCs, including those with EGFR or K-Ras mutations.

Disclosure of Potential Conflicts of Interest

D. Raina, G. Panchamoorthy, and S. Kharbanda are employees of Genus Oncology. D. Kufe is an equity holder and consultant for Genus Oncology.

Acknowledgments

The authors thank Dr. Pasi Janni for the H2228 cells. Genus Oncology provided the peptides used in these experiments and conducted the animal studies at a contract organization.

Grant Support

This study was supported by Lung Cancer Research Foundation and National Cancer Institute grants CA97908 and CA24820. J. Supko received research support from Genus Oncology.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received November 17, 2010; revised March 4, 2011; accepted March 6, 2011; published OnlineFirst March 18, 2011.

References


Molecular Cancer Therapeutics

Dependence on the MUC1-C Oncoprotein in Non–Small Cell Lung Cancer Cells

Deepak Raina, Michio Kosugi, Rehan Ahmad, et al.

*Mol Cancer Ther* 2011;10:806-816. Published OnlineFirst March 18, 2011.

Updated version
Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-10-1050

Supplementary Material
Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2011/03/18/1535-7163.MCT-10-1050.DC1

Cited articles
This article cites 48 articles, 28 of which you can access for free at:
http://mct.aacrjournals.org/content/10/5/806.full#ref-list-1

Citing articles
This article has been cited by 15 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/10/5/806.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.