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MUC1 ONCOPROTEIN IS A DRUGGABLE TARGET IN HUMAN PROSTATE CANCER CELLS

Maya Datt Joshi¹, Rehan Ahmad¹, Li Yin¹, Deepak Raina², Hasan Rajabi¹, Glenn Bubley³, Surender Kharbanda², and Donald Kufe¹

¹ Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

² Genus Oncology, Boston, MA 02118

³ Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

Abstract

Human prostate cancers are dependent on the androgen receptor for their progression. The MUC1 heterodimeric oncoprotein is aberrantly overexpressed in prostate cancers; however, it is not known if MUC1 is of functional importance to these tumors. To assess dependence on MUC1, we synthesized an inhibitor, designated GO-201, that interacts directly with the MUC1-C subunit at its oligomerization domain. Treatment of MUC1-positive DU145 and PC3 prostate cancer cells with GO-201, and not an altered version, resulted in inhibition of proliferation. GO-201 also induced necrotic cell death that was associated with increases in reactive oxygen species, loss of mitochondrial transmembrane potential and depletion of ATP. By contrast, GO-201 had no effect against MUC1-negative LNCaP, CWR22Rv1 and MDA PCa 2b prostate cancer cells. Significantly, GO-201 treatment of DU145 and PC3 xenografts growing in nude mice resulted in complete tumor regression and prolonged lack of recurrence. These findings indicate that certain prostate cancer cells are dependent on MUC1-C for growth and survival and that directly targeting MUC1-C results in their death in vitro and in tumor models.

Keywords

MUC1; AR; prostate cancer; MUC1 inhibitor; tumorigenicity; oncogene addiction

Introduction

The mucin 1 (MUC1) oncoprotein is aberrantly expressed at high levels in human carcinomas (1) and has become an attractive target for the development of anti-cancer agents. However, there have been no available small molecules to date that directly target MUC1. In this regard, MUC1 is heterodimer that consists of N-terminal (MUC1-N) and C-terminal (MUC1-C) subunits (2), and much of the early work focused on MUC1-N, the mucin component. Importantly, however, the transmembrane MUC1 C-terminal subunit (MUC1-C) includes a cytoplasmic domain that is sufficient for transformation (3,4). Moreover, MUC1-C interacts with diverse effectors, such as the epidermal growth factor receptor (5,6), β -catenin (7), p53 (8), I κ B kinase β (9) and NF- κ B p65 (10), that have been linked to transformation. MUC1-C contains a CQC motif in the cytoplasmic domain that is necessary for its oligomerization and thereby targeting of MUC1-C to the nucleus (11). MUC1-C is also targeted to the mitochondrial outer membrane (MOM) in a complex with HSP70/HSP90 that is dependent on formation of MUC1-C oligomers (12–14). Integration of MUC1-C in the MOM blocks stress-induced loss of the mitochondrial transmembrane potential ($\Delta\psi_m$) (12). Consistent with this effect, overexpression of MUC1 as found in human carcinomas blocks the induction of apoptosis and necrosis in the cellular response to DNA damaging agents (12), reactive oxygen species (15,

16), hypoxia (17) and glucose deprivation (18). Based on these observations, a direct inhibitor of MUC1-C oligomerization was found to induce death of human breast cancer cells growing in vitro and as tumor xenografts (19).

MUC1 is overexpressed in about 60% of primary prostate cancers and 90% of lymph node metastases (20,21). Moreover, 86% of MUC1-positive primary prostate tumors were Gleason grade 7 or higher, supporting an association with more aggressive disease (20). Gene expression profiling of human prostate cancers has also shown that *MUC1* is highly expressed in subgroups with aggressive clinicopathologic features and an elevated risk of recurrence (22). Notably, however, there are no reports that MUC1 contributes to the malignant phenotype of prostate cancer cells. Indeed, prostate cancer cells are dependent on androgen receptor (AR) signaling for growth and survival (23). Moreover, progression of prostate cancer, despite treatment to abrogate androgen action, occurs as a result of continued AR activation by mechanisms that include *AR* gene amplification and mutations (23–25). Production of AR ligands by prostate cancer cells (26), alterations in AR coactivators and repressors (27), and interactions with other signaling pathways (28) have also been associated with progression of prostate cancer to castrate resistant disease. The importance of AR signaling for growth of androgen-insensitive prostate cancer cells has been further supported by the targeting of AR function in in vitro and animal tumor models (29,30). These findings have provided the experimental basis for the development of new agents that inhibit AR function (31–33). Aberrant AR regulation has also been linked to activation of Wnt/ β -catenin (34) and NF- κ B (35,36); however, the effects of targeting these pathways on prostate cancer growth and survival are not known.

The present studies demonstrate that GO-201, an inhibitor of MUC1-C oligomerization, induces death of MUC1-positive, but not MUC1-negative, human prostate cancer cells in vitro. The results also show GO-201 is highly effective in the treatment of human prostate cancer xenografts in nude mice.

Materials and Methods

Cell culture

Human LNCaP, DU145, PC3 and CWR22Rv1 prostate cancer cells (ATCC, Rockville, MD) were grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 μ g/ml streptomycin, 100 units/ml penicillin and 2 mM L-glutamine. MDA PCa 2b prostate cancer cells (ATCC) were grown in Ham's modified F12-K medium according to ATCC guidelines. PC3/neo and PC3/AR cells were provided by Dr. Mien-Chie Hung (M.D. Anderson Cancer Center, Houston, TX). The stably transfected PC3 cells were grown in the presence of 400 μ g/ml G418. Cells were treated with the GO-201 or CP-1 peptides (AnaSpec, Inc., San Jose, CA) as described (19). These peptides contain 24 amino acids (Fig. 2A) and were dissolved in PBS before use. Viability was determined by trypan blue exclusion.

Immunoblot analysis

Whole cell and nuclear lysates were prepared as described (11). Soluble proteins were analyzed by immunoblotting with anti-MUC1-C (Ab5; Neomarkers, Fremont, CA), anti-AR (H-280; Santa Cruz Biotechnology, Santa Cruz, CA), anti- β -actin (Sigma, St. Louis, MO) and anti-lamin B (EMD, La Jolla, CA). Reactivity was detected with horseradish peroxidase-conjugated second antibodies and chemiluminescence.

Analysis of cell cycle distribution and cell membrane integrity

Cells were fixed with 80% ethanol and incubated in PBS containing 40 μ g/ml RNase and 40 μ g/ml propidium iodide. Cell cycle distribution and sub-G1 DNA content was determined by

flow cytometry. For assessment of cell membrane integrity, cells were incubated with 1 $\mu\text{g/ml}$ propidium iodide/PBS and then monitored by flow cytometry as described (17,37).

Measurement of ROS levels

Cells were incubated with 5 μM DCFH-DA (Molecular Probes, Eugene OR) for 20 min at 37°C. Fluorescence of oxidized DCF was measured at an excitation wavelength of 480 nm and an emission wavelength of 525 nm.

Analysis of mitochondrial transmembrane potential

Cells were incubated with 50 ng/ml rhodamine 123 (Molecular Probes) in PBS for 30 min at 37°C and then monitored by flow cytometry.

Measurement of ATP levels

ATP levels were measured using an ATP determination kit (Sigma).

Prostate tumor xenograft models

Balb-c nu/nu male mice (Charles River Laboratories, Wilmington, MA), 4–6 weeks old weighing 18–22 grams, were injected with 1×10^7 DU145 or PC3 cells subcutaneously in the flank. When tumors were detectable, the mice were pair matched into control and treatment groups. Each group contained 6–10 mice, each of which was ear tagged and followed throughout the study. PBS (vehicle), GO-201 and CP-1 were administered daily by intraperitoneal injection. Mice were weighed twice weekly. Tumor measurements were performed with calipers. Tumor volume (V) was calculated using the formula $V = L^2 \times W/2$, where L and W are the larger and smaller diameters, respectively. Tumors and sites of tumor implantation were evaluated by H&E staining.

Results

Expression of MUC1-C in human prostate cancer cell lines

To identify models for targeting MUC1-C function in prostate cancer cells, we first assessed levels of MUC1-C expression. Immunoblot analysis of androgen-dependent, AR-positive LNCaP cells demonstrated undetectable MUC1-C expression (Fig. 1A). By contrast, MUC1-C was detectable at high levels in DU145 cells and to a lower extent in PC3 cells, both of which are androgen-independent and have low to undetectable AR expression as compared to LNCaP cells (Fig. 1A). CWR22Rv1 prostate cancer cells express a mutant AR, proliferate in the absence of androgens and are responsive to androgen (38). MDA PCa 2b cells also express AR and are sensitive to androgens (39). Compared to DU145 cells, MUC1-C expression was low to undetectable in both CWR22Rv1 and MDA PCa 2b cells (Fig. 1B). PC3 cells have been stably transfected with an empty vector or one expressing the human AR coding region (40). AR expression in the PC3 cells confers androgen-responsiveness (40). Immunoblot analysis of the PC3/AR cells demonstrated suppression of MUC1-C levels (Fig. 1C). These prostate cancer cells without and with MUC1 expression thus represented models for assessing the selective targeting of MUC1-C function.

GO-201 blocks localization of MUC1-C to the nucleus

MUC1-C forms oligomers through a CQC motif in the cytoplasmic domain (Fig. 2A) and oligomerization is necessary for MUC1-C nuclear transport (11). To block nuclear targeting of MUC1-C, we synthesized a peptide, designated GO-201, containing the CQC motif and a similar control peptide (CP-1) in which CQC was changed to AQA (Fig. 2A). A poly D-arginine protein-transduction domain was included in both peptides to enhance their entry into cells (41). As determined by BIAcore analysis, GO-201 and not CP-1 binds to the MUC1-CD

oligomerization domain with a dissociation constant of 30 nM, which is comparable to that for dimers of the full-length protein (11,19). To assess effects on nuclear targeting of MUC1-C, DU145 cells were treated with 5 μ M GO-201 or CP-1. There was no apparent effect of either agent on MUC1-C levels in whole cell lysates (Fig. 2B, left). However, treatment with GO-201, but not CP-1, was associated with a decrease in nuclear MUC1-C levels (Fig. 2B, right). Similar effects of GO-201 were found when treating PC3 cells (Fig. 2C). These findings indicated that GO-201 is effective in targeting MUC1 function in DU145 and PC3 prostate cancer cells.

GO-201 is a selective inhibitor of MUC1-positive prostate cancer cell growth

Treatment of DU145 cells with GO-201, but not CP-1, was associated with an initial slowing of growth and then a decline in cell number (Fig. 3A). By contrast, GO-201 had no apparent effect on growth of the MUC1-negative LNCaP cells (Fig. 3B). Treatment of PC3 cells with GO-201 also resulted in a slowing of growth and then decline in cell number, a response similar to that in DU145 cells but kinetically less rapid (Fig. 3C, left). Notably, downregulation of MUC1 as found in PC3/AR cells attenuated the growth inhibitory effects of GO-201 compared to that in PC3 cells (Fig. 3C, right). Moreover, GO-201 had little if any effect on growth of the MUC1-negative CWR22Rv1 and MDA PCa 2b cells (Fig. 3D). These results demonstrate that the growth inhibitory effects of GO-201 are selective for MUC1-positive prostate cancer cells.

GO-201 induces death of DU145 and PC3 cells

To further define the effects of GO-201, DU145 cells were treated with GO-201 for 3 d and monitored for cell cycle distribution. GO-201 treatment, but not a similar exposure to CP-1, was associated with an accumulation of cells in S phase, consistent with the growth inhibitory effects (Fig. 4A). GO-201 treatment was also associated with the appearance of cells with substantial DNA degradation (Fig. 4A). Similar results were obtained on day 4 of treatment (Fig. 4A). In addition, treatment with GO-201 and not CP-1 was associated with uptake of propidium iodide, consistent with loss of cell membrane integrity, on days 3 and 4 (Fig. 4B). PC3 cells similarly responded to GO-201 with arrest in S phase (Fig. 4C) and loss of cell membrane integrity (Fig. 4D and Supplemental Fig. S1), although this effect was delayed compared to that in DU145 cells.

GO-201 disrupts redox balance and mitochondrial function

Previous work has shown that MUC1 suppresses disruption of redox balance and that silencing MUC1 is associated with increases in ROS (15–18). To define the basis for GO-201-induced death, cells were thus monitored for changes in ROS levels. Treatment of DU145 cells with GO-201, but not CP-1, was associated with over a 2-fold increase in ROS (Figs. 5A and B). Disruption of redox balance contributes to loss of mitochondrial transmembrane potential ($\Delta\psi$ m) and depletion of ATP (42,43). As determined by rhodamine 123 uptake, GO-201 treatment of DU145 cells resulted in an approximately 50% decrease in $\Delta\psi$ m (Figs. 5C and D, left). Increases in ROS and loss of $\Delta\psi$ m were also observed in the response of PC3 cells to GO-201 (Supplemental Fig. S2A–D, left). Moreover, the demonstration that GO-201 treatment is associated with decreases in ATP levels (Fig. 5D, right and Supplemental Fig. S2D, right) indicated that the extensive DNA degradation and loss of cell membrane integrity is associated with induction of a necrotic death response (37,44).

GO-201 induces complete regressions of DU145 and PC3 tumors

To assess anti-tumor activity, DU145 tumor xenografts (~ 225 mm³) were established in the flanks of nude mice. Administration of GO-201 at 30 mg/kg/d $\times$ 21 d slowed growth as compared to that obtained with vehicle (PBS), whereas CP-1 also given at 30 mg/kg/d $\times$ 21 d had no apparent effect (Fig. 6A, left). Histopathology of DU145 tumors harvested on day 21

demonstrated a predominance of cells with pyknotic nuclei and decreased cytoplasm (Fig. 6A, right).

To assess the long-term effects of targeting MUC1-C, additional groups of 10 mice bearing DU145 tumors were treated with GO-201 and followed for longer periods. Dosing of GO-201 at 30 mg/kg/d $\times$ 21 d and on a different schedule at 30 mg/kg/d $\times$ 5 d/week for 3 weeks was associated with an initial cessation of tumor growth and then a progressive decrease in volume (Fig. 6B, left). Notably, the tumors were no longer palpable by day 40 (Fig. 6B, left). On day 42, one mouse from each group was sacrificed to assess the tumor implantation site. There was no visual evidence for remaining tumor or extension to other organs. Histologic examination of the tumor implantation sites for both GO-201 treatment groups further indicated that there were no remaining tumor cells (Fig. 6B, right and data not shown). The remaining mice in both GO-201 treatment groups are being followed and, as of week 29, there has been no evidence for reappearance of tumors.

Treatment of PC3 tumors ($\sim 200 \text{ mm}^3$) with GO-201 at 30 mg/kg/d $\times$ 21 d or at 30 mg/kg/d $\times$ 5 d each week for 3 weeks had little effect on growth (Fig. 6C, left). Consequently, treatment was continued for an additional week and at the end of dosing there was evidence of growth inhibition for both dose schedules (Fig. 6C, left). Analysis of PC3 tumors on day 28 demonstrated loss of tumor architecture and decreased abundance of tumor cells, as compared to that for control and CP-1-treated tumors (Fig. 6C, right). Importantly and despite having discontinued dosing, tumor regression continued through day 43, at which point tumors that had been treated with both dose schedules were no longer palpable. As of week 18, there has been no evidence for recurrence of the GO-201-treated tumors.

Discussion

GO-201 selectively blocks growth of MUC1-positive prostate cancer cells

MUC1 is overexpressed in primary prostate cancers and predominantly in those with more aggressive disease (20–22,45,46). However, there has been no direct evidence that MUC1 is of importance to prostate cancer cell growth and survival. In the present studies, an inhibitor of MUC1-C, designated GO-201 (19), was used to assess MUC1 dependence of prostate cancer cells. The MUC1-C transforming function is dependent on the formation of oligomers that are mediated by a CQC motif in the cytoplasmic domain (4,11,19). The present results demonstrate that treatment of MUC1-positive DU145 and PC3 cells with GO-201, a direct inhibitor of MUC1 oligomerization (19), is initially associated with inhibition of growth. The anti-proliferative effects of GO-201 were more pronounced for DU145 cells, which express higher levels of MUC1-C as compared to PC3 cells. By contrast, GO-201 had no effect on growth of MUC1-negative LNCaP, CWR22Rv1 or MDA PCa 2b prostate cancer cells. The selectivity of GO-201 was further supported by the absence of an effect on PC3/AR cells, which were found to have downregulation of MUC1 expression and served as an additional control. Decoy peptides derived from the MUC1-C cytoplasmic domain have been used to block interactions between MUC1-C and certain binding partners, such as β -catenin (7,47,48). In this regard, recent studies have shown that the PMIP peptide slows proliferation of human breast cancer cells (48). Unlike decoy peptides, GO-201 binds to the MUC1-C CQC motif and thereby directly blocks MUC1 function. Indeed, the control CP-1, which has an AQA motif, does not bind to MUC1-C and had no effect on growth of MUC1-positive prostate cancer cells.

Inhibiting MUC1-C induces complete regression of MUC1-positive prostate tumors in xenograft models

Redox balance is regulated by MUC1-dependent signaling (15–18). In this context, targeting MUC1-C function with GO-201 in DU145 and PC3 cells was associated with increases in

ROS. GO-201-induced disruption of redox balance was also associated with a substantial loss of $\Delta\psi_m$, consistent with the effects of increased ROS levels on mitochondrial dysfunction (42,43). Notably, MUC1-C localizes to the mitochondrial outer membrane where it attenuates loss of $\Delta\psi_m$ in the response to stress (12–14). Thus, GO-201-induced loss of $\Delta\psi_m$ may have been related to both increases in ROS and disruption of MUC1-C function in the mitochondrial outer membrane. In concert with these responses, targeting of MUC1-C with GO-201 was also associated with depletion of ATP, which is associated with random DNA degradation and loss of cell membrane integrity in necrotic cell death (44). Treatment of DU145 tumors in nude mice similarly responded to GO-201 treatment with an initial slowing of growth. Importantly, after completing treatment, the tumors exhibited cells with pyknotic nuclei and underwent complete regression over the next few weeks. Slowing of PC3 tumor growth was evident at 28 days of GO-201 treatment and after discontinuing dosing, the tumors also regressed completely. Significantly, there has been no evidence for recurrence of the DU145 and PC3 tumors as of weeks 29 and 18, respectively. These prolonged effects of directly inhibiting MUC1-C with GO-201 are consistent with those observed for the treatment of human breast tumor xenografts (19).

Are human prostate cancer cells addicted to MUC1-C function?

The present work provides support for the importance of MUC1-C in supporting the growth and survival of MUC1-positive prostate cancer cells. However, unlike AR, which is the active focus for development of new agents against prostate cancer (31–33), there has been no previous evidence that MUC1 also represents a potential target for this disease. The present results do not exclude the possibility that MUC1-C interacts directly or indirectly with AR signaling and thereby contributes to the growth and survival of prostate cancer cells. For example, β -catenin is a coregulator of AR-mediated transcription (34,49,50). MUC1-C blocks β -catenin degradation by a direct interaction (4,7), and by extension could contribute to AR signaling. Castrate-resistant prostate cancers have also been linked to constitutive activation of the IKK- $\rightarrow$ NF- κ B pathway (24,35,36). Notably, MUC1-C interacts with both IKK β and RelA, and contributes to constitutive NF- κ B activation (9,10). Therefore, it is formally possible that MUC1-C participates in AR signaling and that of other effectors involved in prostate cancer progression. Based on the present findings, one can conclude that the MUC1-C CQC oligomerization motif is indeed an Achilles' heel of the MUC1 oncoprotein and that the prostate cancer cells studied here are dependent on MUC1 function. Patients with prostate cancers that overexpress MUC1 may therefore be candidates for treatment with MUC1-C oligomerization inhibitors in the form of peptides for systemic delivery or small molecules for oral administration. Further studies are thus warranted to determine whether this direct targeting of MUC1-C is associated with the emergence of circumventing MUC1 mutations or cells that lose dependency on MUC1 for growth and survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MUC1   mucin 1

MUC1-N	MUC1 N-terminal subunit
MUC1-C	MUC1 C-terminal subunit, MUC1-CD, MUC1 cytoplasmic domain
AR	androgen receptor
ROS	reactive oxygen species
MOM	mitochondrial outer membrane
$\Delta\psi_m$	mitochondrial transmembrane potential

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Figure 1. Expression of MUC1-C and AR in prostate cancer cell lines

A and B. Lysates from human LNCaP, PC3, DU145, CWR22Rv1 and MDA PCa 2b cells were immunoblotted with the indicated antibodies. C. Lysates from PC3 cells stably expressing an empty vector (PC3/Neo) or AR (PC3/AR) were immunoblotted with the indicated antibodies.

Figure 2. GO-201 downregulates nuclear MUC1-C levels

A. Amino acid sequence of the 72 amino acid MUC1-CD (upper panel). The N-terminal 15 amino acid (shaded sequence) GO-201 and mutated (CQC->AQA) CP-1 peptides were synthesized with the d-Arg transduction domain. B and C. DU145 (B) and PC3 (C) cells were left untreated (Control) and treated with 5 μ M GO-201 or CP-1 each day for 3 d. Whole cell lysates (WCL) (left) and nuclear lysates (right) cells were immunoblotted with the indicated antibodies.

Figure 3. GO-201 selectively inhibits growth of MUC1-positive prostate cancer cells

A. DU145 cells were left untreated (triangles) and treated with 5 μ M GO-201 (squares) or CP-1 (diamonds) each day for 6 d. Viable cell numbers were determined by trypan blue exclusion. B–D. LNCaP (B), PC3 (C, left), PC3/AR (C, right), CWR22Rv1 (D, left) and MDA PCa 2b (D, right) cells were left untreated (open bars) and treated with 5 μ M GO-201 (solid bars) or CP-1 (shaded bars) each day for the indicated days. Viable cell numbers were determined by trypan blue exclusion. The results are expressed as the mean of three determinations with a SD of <10%.

A. DU145 **B. DU145**

Figure 4. GO-201 induces cell cycle arrest and death

A. and B. DU145 cells were left untreated and treated with 5 μ M GO-201 or CP-1 each day for 3 and 4 days. Cells were fixed and analyzed for cell cycle distribution by flow cytometry (A). The percentage of cells in S phase is included in the panels. Cells were stained with propidium iodide and analyzed by flow cytometry (B). The percentage of cells with loss of cell membrane integrity is included in the panels. C. PC3 cells were left untreated and treated with 5 μ M GO-201 or CP-1 each day for 4 days. Cells were fixed and analyzed for cell cycle distribution by flow cytometry. The percentage of cells in S phase is included in the panels. D. PC3 cells were left untreated and treated with 5 μ M GO-201 or CP-1 each day for 10 days. Cells were stained with propidium iodide and analyzed by flow cytometry. The percentage of cells with loss of cell membrane integrity is included in the panels.

A. DU145/ROS **B. DU145** **C. DU145/ $\Delta\psi_m$** **D. DU145** **Figure 5. GO-201 increases ROS and induces loss of $\Delta\psi_m$ and depletion of ATP**

A–D. DU145 cells were left untreated and treated with 5 μM GO-201 or CP-1 each day for 3 days. Cells were incubated with DCFH-DA and fluorescence of oxidized DCF was measured by flow cytometry (A). The results are expressed as the relative ROS level (mean $\pm$ SD of three determinations) compared to that obtained for the control (B). Cells were incubated with rhodamine 123 and analyzed by flow cytometry (C). The results are expressed as the percentage $\Delta\psi_m$ (mean $\pm$ SD of three determinations) compared to that obtained for the control (D, left). ATP levels are expressed as the mean $\pm$ SD of six determinations relative to that of the control (D, right).

Figure 6. GO-201 induces regression of DU145 and PC3 tumors

A. Four to six week old male Balb-c nu/nu mice were injected subcutaneously in the flank with 1×10^7 DU145 cells. When tumors were ~ 225 mm³ (range: 200–275 mm³) the mice were pair matched into groups of 6 and injected intraperitoneally with PBS (vehicle control; squares), 30 mg/kg GO-201 each day for 21 d (triangles) or 30 mg/kg CP-1 each day for 21 d (circles). Results are expressed as the mean tumor volume with a SD of <15% (left). There was no evidence of weight loss in any of the groups. Tumors harvested on day 21 from the control, GO-201 and CP-1 treatment groups were stained with H&E (right). B. Male Balb-c nu/nu mice were injected subcutaneously in the flank with 1×10^7 DU145 cells. When tumors were 175–250 mm³ the mice were pair matched into groups of 10 and injected intraperitoneally with PBS (closed squares), 30 mg/kg GO-201 each day for 21 d (closed triangles) or 30 mg/kg GO-201 each day for 5 d/week $\times$ 3 weeks (open circles) (left). There was no evidence of weight loss in any of the groups. Control DU145 tumor harvested on day 34 and the DU145 implantation site from mice treated with GO-201 each day for 5 d/week $\times$ 3 weeks obtained on day 42 were stained with H&E (right). C. Male Balb-c nu/nu mice were injected subcutaneously in the flank with 1×10^7 PC3 cells. The mice were pair-matched into groups of 10 when the tumors reached ~ 200 mm³ (range: 175–250 mm³). The mice were injected intraperitoneally with PBS (squares), 30 mg/kg GO-201 each day $\times$ 21 d (closed circles), 30 mg/kg GO-201 each day for 5 d/week $\times$ 3 weeks (open circles) or 30 mg/kg CP-1 each day $\times$ 21 d (triangles) (left). There was no weight loss in any of the groups. Tumors were harvested on day 28 and stained with H&E (right).