Intracellular Targeting of the Oncogenic MUC1-C Protein with a Novel GO-203 Nanoparticle Formulation

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Abstract

Purpose: The MUC1-C oncoprotein is an intracellular target that is druggable with cell-penetrating peptide inhibitors. However, development of peptidyl drugs for treating cancer has been a challenge because of unfavorable pharmacokinetic parameters and limited cell-penetrating capabilities.

Experimental Design: Encapsulation of the MUC1-C inhibitor GO-203 in novel polymeric nanoparticles was studied for effects on intracellular targeting of MUC1-C signaling and function.

Results: Our results show that loading GO-203 into tetrablock polylactic acid (PLA)-polyethylene glycol (PEG)-polypropylene glycol (PPG)-PEG copolymers is achievable and, notably, is enhanced by increasing PEG chain length. In addition, we found that release of GO-203 from these nanoparticles is controllable over at least 7 days. GO-203/nanoparticle treatment of MUC1-C-positive breast and lung cancer cells in vitro was more active with less frequent dosing than that achieved with nonencapsulated GO-203. Moreover, treatment with GO-203/nanoparticles blocked MUC1-C homodimerization, consistent with on-target effects. GO-203/nanoparticle treatment was also effective in downregulating TIGAR, disrupting redox balance, and inhibiting the self-renewal capacity of cancer cells. Significantly, weekly administration of GO-203/nanoparticles to mice bearing syngeneic or xenograft tumors was associated with regressions that were comparable with those found when dosing on a daily basis with GO-203.

Conclusions: These findings thus define an effective approach for (i) sustained administration of GO-203 in polymeric PLA-(PEG-PPG-PEG) nanoparticles to target MUC1-C in cancer cells and (ii) the potential delivery of other anticancer peptide drugs.

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Translational Relevance

Intracellular cancer targets devoid of a kinase domain are often undruggable with small-molecule inhibitors. In this context, anticancer peptides, such as GO-203, which inhibits the MUC1-C oncoprotein, are an alternative class of agents for targeting intracellular proteins. The development of anticancer peptide drugs, however, has been limited by their immunogenicity, short circulating half-life, and poor cell penetration. The present work has studied the incorporation of GO-203 into nanoparticles comprised of a novel PLA-PEG-PPG-PEG tetrablock copolymer. Our results demonstrate that these nanoparticles are highly effective for the encapsulation and then release of GO-203 over sustained periods of time. Our findings further demonstrate that this nanoparticle-based approach for intracellular delivery of GO-203 and thereby targeting of MUC1-C induces anticancer activity in vitro and in vivo. These results may also be broadly applicable to the development of other anticancer peptides.

Materials and Methods

Preparation of GO-203-loaded nanoparticles

Polymeric acid (PLA)-polyethylene glycol (PEG)-polypropylene glycol (PPG)-PEG tetrablock copolymers were synthesized as described (25). The nanoparticles were loaded with GO-203 (15) and filtered through an Amikon 10-kDa ultrafilter (Millipore). The filtrate was collected and analyzed for free GO-203 peptide using a Micro-BCA kit (Pierce Chemicals). Encapsulation efficiency, particle size, and zeta potential were determined as described (25).

Assessment of GO-203 release from nanoparticles

The in vitro release kinetics of GO-203 from nanoparticles were determined by the ultrafiltration method as described (25).

Cell culture

ZR-75-1 breast cancer, and H1975 and H460 lung cancer cells (ATCC) were grown in RPMI1640 media supplemented with 10% heat-inactivated FBS (HI-FBS), 100 μg/mL streptomycin, and 100 units/mL penicillin. MCF-7 and MDA-MB-468 breast cancer cells, and HCT116 colon cancer cells (ATCC) were cultured in DMEM media supplemented with HI-FBS and antibiotics. BT-20 breast cancer cells (ATCC) were grown in EMEM media containing HI-FBS and antibiotics. Cells were treated with the MUC1-C inhibitor GO-203 (15) encapsulated in PLA-(PEG-PPG-PEG)\textsubscript{12,5K} nanoparticles (GO-203/nanoparticles), the control peptide CP-2 (15) encapsulated in PLA-(PEG-PPG-PEG)\textsubscript{12,5K} nanoparticles (CP-2/nanoparticles) or unloaded, empty nanoparticles.

Analysis of cell viability

Cells were seeded on 96-well plates in 100 μL growth medium at a density of 1,000 to 2,000 cells per well. After 24 hours, the cells were exposed to one treatment of nanoparticles on day 0 or two treatments of nanoparticles on days 0 and 3. Cell viability was determined in triplicates using the Alamar blue assay on day 3 or 7. Statistical significance between treatment groups was assessed using the Student’s t test.

Immunoblot analysis

Cell lysates were prepared as described (15). Soluble proteins were analyzed by immunoblotting with anti-MUC1-C (30), anti-TIGAR (Abcam), anti-phospho-p38, anti-p38 (Cell Signaling Technologies), or anti–β-actin (Sigma) as described (15). Immune complexes were detected with horseradish peroxidase–conjugated secondary antibodies and enhanced chemiluminescence (PerkinElmer).

Measurement of NADPH and GSH levels

Intracellular NADPH and glutathione (GSH) levels were measured using the EnzyChrom NADP/NADPH Assay Kit (BioAssay Systems) and the Bioxytech GSH-400 Kit (OXIS International), respectively.

Colonies formation assays

Cells were seeded in 6-well plates for 24 hours and then left untreated or treated with nanoparticles. After 10 to 20 days, the cells were washed and stained with 0.5% crystal violet in 25% methanol. Colonies >30 cells were counted in triplicate wells.

Others. However and as noted above, adequate delivery of peptides to tumors can be challenging because of unfavorable pharmacokinetic parameters (16). Administration of peptide drugs can also be limited by their proteolytic degradation or the induction of immune responses. Accordingly, improved delivery systems are needed, at least in part, for the successful development of anticancer peptides. Nanoparticles have been employed to improve the pharmacokinetic properties and therapeutic indices of small-molecule anticancer agents, such as doxorubicin and paclitaxel (22). In this way, nanoparticles have the capacity to sustain drug exposure in the tumor microenvironment by the enhanced permeation and retention (EPR) effect (23). In addition, nanoparticles can be modified with ligands that bind to targets selectively expressed on the surface of tumor cells (24). Among different classes of nanoparticles, polymeric acid (PLA)-polyethylene glycol (PEG) block copolymer nanoparticles are nontoxic and biodegradable (24). In addition, the polymeric PLA-PEG nanoparticles have been administered clinically for the delivery of small-molecule anticancer agents (24). By contrast and to our knowledge, polymeric nanoparticles have not been explored for the delivery of anticancer peptides, other than in a recent preclinical study (25). In this regard, anticancer peptides have been incorporated into cationic liposomes (26), perfluorocarbon nanoemulsion vehicles (27), cyclodextrin polymerized nanoparticles (28), and liposome–protamine–heparin nanoparticles (29). However, unlike polymeric nanoparticles, these nanoparticle formulations have evidently not been advanced for clinical applications.

The present studies have investigated the encapsulation of GO-203 into polymeric tetrablock nanoparticles as a delivery system to target the oncogenic MUC1-C protein in cancer cells. The results demonstrate that the delivery of GO-203 in nanoparticles is an effective approach for inhibiting intracellular MUC1-C homodimerization and function. Our findings thus provide support for the further development of GO-203/nanoparticles as a therapeutic for the treatment of MUC1-C–expressing malignancies.
were injected subcutaneously with 5 nanoparticle formulations for 3 weeks. Four- to 6-week-old Balb/c nu/nu mice were randomized into groups of 6 mice each and treated i.p. (i) each day with vehicle control or 15 mg/kg GO-203 for 21 days, or (ii) once each week with 10, 15, or 20 mg/kg GO-203 for 21 days, or (i) each day with vehicle control or 18 mg/kg GO-203 for 21 days, or (ii) once each week with vehicle control or 20 mg/kg GO-203/nanoparticles for 3 weeks. Tumors were measured every other day with calipers, and tumor volumes were calculated using the formula \( V = \frac{1}{2} l \cdot w^2 \), where \( l \) and \( w \) are the longest and shortest tumor diameters, respectively. Statistical analysis of tumor volumes was performed by one-way ANOVA and the Dunnett test using Origin 8.0 (Origin Lab). Treatment with GO-203/nanoparticles decreases survival of breast cancer cells GO-203 is effective in inducing death of MUC1-expressing breast and other types of cancer cells (3, 15, 33, 34). To determine whether encapsulated GO-203 is also effective in inhibiting cell viability, we first treated ZR-75-1 breast cancer cells with 7.5 \( \mu \)mol/L GO-203/nanoparticles. In these studies, a single exposure to GO-203/nanoparticles on day 0 was associated with loss of survival as detected on day 3 (Fig. 2A, left). By contrast,
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Effects of GO-203/nanoparticles (NP) on viability of breast cancer cells. A, ZR-75-1 cells were treated with 7.5 μmol/L GO-203/nanoparticles or an equal amount of empty nanoparticles on day 0 (left). The results are expressed as a percentage of cell death (mean ± SD of 3 replicates) as determined by Alamar blue analysis on day 3. MDA-MB-468 cells were treated with 7.5 μmol/L GO-203/nanoparticles or an equal amount of empty nanoparticles on day 0, or 2.5 μmol/L GO-203 peptide each day for 3 days (right). The results are expressed as a percentage of cell death (mean ± SD of 3 replicates) on day 3. B, ZR-75-1 cells were treated with 2.5 or 3.3 μmol/L GO-203/nanoparticles once on day 0 (left). The results are expressed as a percentage of cell death (mean ± SD of 3 replicates) on day 3. MDA-MB-468 cells were treated with 2.5 μmol/L GO-203/nanoparticles or 2.5 μmol/L GO-203 on days 0 and 3 (left). The results are expressed as a percentage of cell death (mean ± SD of 3 replicates) on day 3. C, BT-20 cells were treated with 7.5 or 10 μmol/L GO-203/nanoparticles or CP-2/nanoparticles once on day 0. BT-20 cells were also treated with 2.5 or 3.3 μmol/L GO-203 peptide each day for 3 days. The results are expressed as a percentage of cell death (mean ± SD of 3 replicates) on day 3. D, HCT116 cells were treated with 7.5 or 15 μmol/L GO-203/nanoparticles or CP-2/nanoparticles once on day 0. HCT116 cells were also treated with 15 μmol/L GO-203 once on day 0 or 5 μmol/L GO-203 peptide each day for 3 days. The results are expressed as a percentage of cell death (mean ± SD of 3 replicates) on day 3.

GO-203/nanoparticles disrupt MUC1-C homodimerization and disrupt redox balance in breast cancer cells

Targeting MUC1-C with unencapsulated GO-203 induces death of carcinoma cells by blocking MUC1-C homodimerization and thereby disrupting redox balance (13, 15). To determine whether delivery of GO-203 in nanoparticles induces similar effects, we first treated ZR-75-1 cells with GO-203/nanoparticles and monitored effects on MUC1-C homodimerization. As found with unencapsulated GO-203 (13), treatment with GO-203/nanoparticles was associated with inhibition of MUC1-C homodimer formation (Fig. 3A), indicating that delivery of GO-203 in nanoparticles to these carcinoma cells results in MUC1-C targeting. MUC1-C promotes expression of the p33-inducible regulator of glycolysis and apoptosis (TIGAR, ref. 35), which shunts glucose-6-phosphate into the pentose phosphate pathway (PPP; ref. 36). Consistent with targeting MUC1-C, we found that treatment of ZR-75-1 cells with GO-203/nanoparticles is associated with suppression of TIGAR levels (Fig. 3B). Moreover, addition of the antioxidant N-acetylcysteine (NAC) blocked GO-203/nanoparticle-induced TIGAR suppression (Fig. 3B), in concert with a ROS-mediated mechanism. TIGAR promotes the production of NADPH and GSH by the PPP (36). In this way, targeting MUC1-C with GO-203/nanoparticles was also associated with marked decreases in NADPH (Fig. 3C, left) and GSH (Fig. 3C, right).
levels. Moreover, and consistent with these results, we found that treatment with GO-203/nanoparticles, but not CP-2/nanoparticles, results in induction of phospho-p38 (Fig. 3D), which is activated in response to disruption of redox balance (37).

GO-203/nanoparticles are effective against non–small cell lung cancer cells.

Non–small cell lung cancer (NSCLC) cells are also sensitive to GO-203 treatment (15). Accordingly, we asked if NSCLC cells respond similarly to GO-203/nanoparticles. Indeed, GO-203/nanoparticle treatment of H1975 NSCLC cells, which harbor the EGFR(L858R/T790M) mutations, was associated with loss of viability (Fig. 4A, left). Other studies performed with H460/KRAS(Q61H) NSCLC cells further demonstrated that GO-203/nanoparticles are active in the setting of mutant KRAS expression (Fig. 4A, right). By contrast, exposure of H1975 and H460 NSCLC cells to empty nanoparticles was associated with substantially lower levels of cell death that were not significantly different from those obtained for ZR-75-1 and MDA-MB-468 breast cancer cells (Fig. 2A, left and right). As shown for breast cancer cells, we also found that treatment of H1975 cells with GO-203/nanoparticles is associated with disruption of MUC1-C homodimers (Fig. 4B, left). Moreover, GO-203 treatment resulted in downregulation of TIGAR (Fig. 4B, right) and decreases in NADPH (Fig. 4C, left) and GSH (Fig. 4C, right) levels. Treatment of H1975 cells with GO-203/nanoparticles was also associated with activation of p-p38 (Fig. 4D), confirming that delivery of GO-203 in nanoparticles is effective in disrupting redox balance.

GO-203/nanoparticles inhibit breast and lung cancer cell self-renewal

Disruption of redox balance inhibits the capacity of cancer cells to undergo self-renewal (38). Accordingly, we first investigated the effects of GO-203/nanoparticles on clonogenic survival of ZR-75-1 cells. Treatment with the GO-203/nanoparticles, but not the empty nanoparticles, was highly effective in inhibiting colony formation (Fig. 5A, left). Similar results were obtained with GO-203/nanoparticle treatment of MDA-MB-468 (Fig. 5A, middle) and MCF-7 (Fig. 5A, right) cells, indicating that GO-203/nanoparticle exposure is sufficient to inhibit clonogenic survival of breast cancer cells. The formation of spheres under nonadherent conditions and in the absence of serum selects for the growth of self-renewing cancer stem–like cells (CSC) that survive anoikis (39, 40). Therefore, to determine if the GO-203/nanoparticles are active against self-renewing populations, MDA-MB-468 cells were treated with GO-203/nanoparticles or CP-2/nanoparticles for 48 hours and then grown in mammosphere medium in the absence of nanoparticles. As expected from previous studies (9), the control MDA-MB-468 cells formed mammospheres (Fig. 5B). Moreover, treatment with GO-203/nanoparticles, but not CP-2/nanoparticles, was highly effective in inhibiting mammosphere formation and decreasing SFE (Fig. 5B). GO-203/nanoparticles also inhibited MCF-7 mammosphere formation (Fig. 5C).
supporting the activity of GO-203/nanoparticles against self-renewing breast cancer cells.

In investigating the effects of GO-203/nanoparticles against clonogenic NSCLC cell populations, we found that GO-203/nanoparticles completely inhibited H1975 (Supplementary Fig. S1A) and H460 (Supplementary Fig. S1B) colony formation. Moreover, GO-203/nanoparticle treatment blocked growth of H1975 (Supplementary Fig. S1C) and H460 (Supplementary Fig. S1D) cells as spheres, indicating that encapsulation of GO-203 in nanoparticles is effective in inhibiting self-renewal of NSCLC cells.

GO-203/nanoparticles inhibit tumor growth

To investigate whether GO-203/nanoparticles are effective in inhibiting growth of human tumor xenografts in nude mice, we first performed studies in Balb/c mice bearing established subcutaneous syngeneic Muc1-positive Ehrlich breast tumors. In this model and consistent with previous studies of human tumor xenograft models (15), treatment with nonencapsulated GO-203 was associated with inhibition of Ehrlich tumor growth (Fig. 6A). Based on the kinetics of GO-203 release from the nanoparticles over 7 days (Fig. 1C), we treated the Ehrlich tumor–bearing mice i.p. once a week for 3 weeks with doses of 10, 15, or 20 mg/kg GO-203/nanoparticles (Fig. 6B). As compared with the control mice, treatment with 10 mg/kg GO-203/nanoparticles was associated with partial slowing of Ehrlich tumor growth (Fig. 6B). By contrast, i.p. dosing of 15 and 20 mg/kg GO-203/nanoparticles resulted in complete inhibition of Ehrlich tumor growth, supporting a dose–response effect (Fig. 6B). In previous studies of nude mice bearing subcutaneous NSCLC xenografts, treatment with nonencapsulated 30 mg/kg GO-203 i.p. daily for 21 days was associated with complete regressions (15). In the present work, i.p. administration of GO-203 each day at a dose of 18 mg/kg was also effective in the treatment of ZR-75-1 xenografts (Fig. 6C). Notable findings in these experiments were that treatment with GO-203 on a weekly or twice-weekly schedule had little, if any, effect on ZR-75-1 tumor growth, indicating that frequent (daily) dosing is necessary for activity (data not shown). By comparison, weekly treatment of ZR-75-1 xenografts with GO-203/nanoparticles at a dose of 20 mg/kg was highly effective in inducing tumor regressions (Fig. 6D). Significantly, there was no overt evidence of toxicity, such as weight loss, associated with GO-203/nanoparticle administration. These findings thus support the notion that delivery of GO-203 in nanoparticles requires less frequent administration and lower total doses than that needed for equivalent antitumor activity when treating with nonencapsulated GO-203.

Discussion

The oncogenic MUC1-C protein is aberrantly expressed in carcinomas of the breast, lung, and other epithelia (1, 5). In addition, recent evidence has supported a role for MUC1-C in promoting the epithelial–mesenchymal transition and other characteristics of CSCs (8, 9). These findings have collectively provided the rationale for developing therapeutic approaches that target MUC1-C function. The present studies have identified a novel strategy for targeting MUC1-C in carcinoma cells by...
intracellular delivery of the peptidyl MUC1-C inhibitor, GO-203, in polymeric nanoparticles. Cell-penetrating peptides have emerged as promising therapeutics because of their potential for targeting intracellular proteins that lack hydrophobic pockets and thereby are often undruggable with small molecules. Moreover, peptidyl drugs have the potential for inhibiting intracellular proteins with greater specificity and less off-target toxicity than small molecules (41, 42). Nonetheless, the development of peptidyl drugs can be hampered by poor pharmacologic properties, such as short circulating half-lives that require frequent administration (16). Therapeutic peptides can also be limited by extracellular degradation and inefficient cell-penetrating capabilities. Accordingly, the present work first investigated whether GO-203 could indeed be loaded into nanoparticles.

There is presently limited information about the optimal conditions for encapsulating peptides into polymeric nanoparticles, and the copolymers used will likely vary depending on the peptidyl drug itself. The approach we used in the present work was based upon a recent experience in which we encapsulated the anticancer peptide NuBCP-9 into tetrablock PLA-(PEG-PPG-PEG)12.5K copolymers (25). The PLA-PEG copolymer is a bilayer structure with a PLA hydrophobic core and a PEG hydrophilic shell interacting with the aqueous medium. The importance of the tetrablock structure is that conjugation of PEG-PPG-PEG with PLA extends the hydrophobic core and thereby has the capacity for increasing peptide uptake and sustaining its release (25). The PLA-PEG-PPG-PEG formulation was thus selected based on the findings demonstrating that (i) peptide encapsulation is greater than that obtained with PLA, and (ii) peptide release is more sustained compared with that found for the PLA-PEG nanoparticles (25). Indeed, as compared with PLA-based nanoparticles, we found that loading of GO-203 is increased with PLA-PEG-PPG-PEG nanoparticles. We also found that approximately 50% of the GO-203 encapsulated into PLA-PEG-PPG-PEG nanoparticles is released over 7 days at physiologic pH, which appeared to be favorable kinetics when considering that an objective of delivering GO-203 in nanoparticles to cancer cells would be prolonged intracellular exposure of endogenous MUC1-C to this agent.

Nonencapsulated GO-203 is effective in inducing death of MUC1-positive carcinoma cells in vitro when low micromolar concentrations are added each day to the medium over 4 to 6 days (15). Importantly, the present studies show that encapsulation of GO-203 in nanoparticles is also effective in killing breast and lung cancer cells, indicating that loading of GO-203 into nanoparticles is not associated with loss of activity. Indeed, we found that treatment with the GO-203/nanoparticles is even more effective than that obtained with nonencapsulated GO-203 at equivalent doses. Moreover, delivery of GO-203 in nanoparticles required...

Figure 5. Effects of GO-203/nanoparticles (NP) on breast cancer cell self-renewal. A, the indicated breast cancer cells were seeded at 1000 cells/well in 6-well plates and left untreated (control) or treated with 7.5 μmol/L GO-203/nanoparticles or an equal amount of empty nanoparticles on day 0. After 3 days, nanoparticle-containing media were replaced with fresh media. Colonies were stained with crystal violet on day 20 after treatment (top). Colony number (>30 cells) is expressed as the mean ± SD of 3 replicates (bottom). B, MDA-MB-468 cells were left untreated (control) or treated with 7.5 μmol/L GO-203/nanoparticles or CP-2/nanoparticles once on day 0. Representative images are shown for the indicated MDA-MB-468 cells grown for 5 days after treatment in sphere culture (top). Bar, 100 μm. The percentage SFE is expressed as the mean ± SD of 3 determinations (bottom). C, MCF-7 cells were left untreated (control) or treated with 7.5 μmol/L GO-203/nanoparticles or CP-2/nanoparticles once on day 0. The cells were then plated at 2,000 cells/well in sphere culture on day 2. Representative images are shown for the indicated MCF-7 cells grown for 5 days after treatment in sphere culture (top). Bar, 100 μm. The percentage SFE is expressed as the mean ± SD of 3 determinations (bottom).
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Antitumor activity of GO-203/nanoparticles (NP). A and B, Balb/c mice (10 per group) with subcutaneous Ehrlich tumors (~40 mm³) were treated IP with (A) vehicle control (closed squares) or 15 mg/kg GO-203 (closed circles) each day for 21 days, and (B) vehicle control (closed squares), 10 mg/kg (closed circles), 15 mg/kg (open circles) or 20 mg/kg (closed triangles) GO-203/nanoparticles once a week for 3 weeks. Tumor volumes were determined on the indicated days of treatment. The results are expressed as tumor volumes (mean ± SEM for 10 mice). C and D, nude mice (6 per group) with subcutaneous ZR-75-1 tumors (~100 mm³) were treated IP with (C) vehicle control (closed squares) or 18 mg/kg GO-203 each day for 21 days, and (D) vehicle control (closed squares) or 20 mg/kg GO-203/nanoparticles (closed circles) once a week for 5 weeks. Tumor volumes were determined on the indicated days of treatment. The results are expressed as tumor volumes (mean ± SEM for 6 mice).

Figure 6. Antitumor activity of GO-203/nanoparticles (NP). A and B, Balb/c mice (10 per group) with subcutaneous Ehrlich tumors (~40 mm³) were treated IP with (A) vehicle control (closed squares) or 15 mg/kg GO-203 (closed circles) each day for 21 days, and (B) vehicle control (closed squares), 10 mg/kg (closed circles), 15 mg/kg (open circles) or 20 mg/kg (closed triangles) GO-203/nanoparticles once a week for 3 weeks. Tumor volumes were determined on the indicated days of treatment. The results are expressed as tumor volumes (mean ± SEM for 10 mice). C and D, nude mice (6 per group) with subcutaneous ZR-75-1 tumors (~100 mm³) were treated IP with (C) vehicle control (closed squares) or 18 mg/kg GO-203 each day for 21 days, and (D) vehicle control (closed squares) or 20 mg/kg GO-203/nanoparticles (closed circles) once a week for 5 weeks. Tumor volumes were determined on the indicated days of treatment. The results are expressed as tumor volumes (mean ± SEM for 6 mice).

However, the experience with nanoparticle delivery of anticancer peptides remains limited. The present results demonstrate that loading of GO-203 into polymeric nanoparticles markedly improves the delivery of GO-203 as an anticancer agent in vivo.

In this way, the anticancer activity of nonencapsulated GO-203 requires daily administration to sustain tumor exposure, consistent with a short circulating half-life in mice (15). By comparison, administration of GO-203/nanoparticles once weekly was sufficient to confer a similar level of antitumor activity to that achieved with daily delivery of GO-203. The marked enhancement of antitumor activity found with GO-203/nanoparticles could be attributed in part to the EPR effect. In addition, uptake of GO-203/nanoparticles by tumor cells and the sustained release of GO-203, as observed over 7 days at physiologic pH, likely also contribute to more prolonged effects on intracellular targeting of MUC1-C function. These findings with GO-203/nanoparticles are in concert with the recent demonstration that in vivo anticancer activity of the NuBCP-9 peptide is markedly increased by delivery in polymeric nanoparticles, suggesting that this nanoparticle approach may be broadly applicable for other peptides that target intracellular effectors. Polymeric nanoparticles have been widely studied, and certain formulations have been found to be nontoxic and biodegradable (24, 31). For example, with degradation of PLA from the PLA-PEG-PLG-PEG nanoparticles used in the present work, the resulting PEG-PLG block (~12.5 kDa) is a nontoxic polymer that is an FDA-approved biomaterial for clinical use (44). With regard to polymeric nanoparticles already in the clinic, Genexol-Pm is a paclitaxel-loaded PLA-PEG–based nanoparticle that is undergoing phase II evaluation for the treatment of metastatic cancers (24, 32). Moreover, BIND Biosciences is evaluating docetaxel-encapsulated biodegradable polymeric nanoparticles for the treatment of patients with solid tumors (24). These ongoing findings with GO-203/nanoparticles are in concert with the recent demonstration that in vivo anticancer activity of the NuBCP-9 peptide is markedly increased by delivery in polymeric nanoparticles, suggesting that this nanoparticle approach may be broadly applicable for other peptides that target intracellular effectors. Polymeric nanoparticles have been widely studied, and certain formulations have been found to be nontoxic and biodegradable (24, 31). For example, with degradation of PLA from the PLA-PEG-PLG-PEG nanoparticles used in the present work, the resulting PEG-PLG block (~12.5 kDa) is a nontoxic polymer that is an FDA-approved biomaterial for clinical use (44). With regard to polymeric nanoparticles already in the clinic, Genexol-Pm is a paclitaxel-loaded PLA-PEG–based nanoparticle that is undergoing phase II evaluation for the treatment of metastatic cancers (24, 32). Moreover, BIND Biosciences is evaluating docetaxel-encapsulated biodegradable polymeric nanoparticles for the treatment of patients with solid tumors (24).
clinical trials with polymeric nanoparticles and the present results thus provide support for the further development of GO-203/nanoparticles as a potential therapeutic agent. In this respect, a phase I trial of nonencapsulated GO-203 administered on a daily basis has been completed, and a MTD has been defined for phase II trials. Our findings here thus lend credence to the notion that GO-203 could be delivered in additional phase II studies on a less frequent schedule and at potentially lower total doses by encapsulation in polymeric nanoparticles.

Disclosure of Potential Conflicts of Interest

D. Kufe is an employee of, has ownership interest (including patents) in, and is a consultant/advisory board member for Genus Oncology. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions

Conception and design: M. Hasegawa, H. Singh, S. Kharbanda, D. Kufe
Development of methodology: M. Hasegawa, R.K. Sinha, M. Kumar, M. Alam, L. Yin, H. Singh

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