RAPID COMMUNICATION

Degradable polymeric nanocapsule for efficient intracellular delivery of a high molecular weight tumor-selective protein complex

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Summary The development of stimuli-responsive, nano-scale therapeutics that selectively target and attack tumors is a major research focus in cancer nanotechnology. A potent therapeutic option is to directly arming the cancer cells with apoptotic-inducing proteins that are not affected by tumoral anti-apoptotic maneuvers. The avian virus-derived apoplin forms a high-molecular weight protein complex that selectively accumulates in the nucleus of cancer cell to induce apoptotic cell death. To achieve the efficient intracellular delivery of this tumor-selective protein in functional form, we synthesized degradable, sub-100 nm, core−shell protein nanocapsules containing the 2.4MDa apoplin complexes. Recombinant apoplin is reversibly encapsulated in a positively charged, water soluble polymer shell and is released in native form in response to reducing conditions such as the cytoplasm. As characterized by confocal microscopy, the nanocapsules are efficiently internalized by mammalian cells lines, with accumulation of rhodamine-labeled apoplin in the nuclei of cancer cells only. Intracellularly released apoplin induced tumor-specific apoptosis in several cancer cell lines and inhibited tumor growth in vivo, demonstrating the potential of this polymer–protein combination as an anticancer therapeutic.
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Abbreviations: NC, nanocapsule; APO, apoplin; S−S, disulfide bonded; AAm, acrylamide; APMAAm, N-(3-aminopropyl)methacrylamide; MBP, maltose binding protein; Rh, rhodamine; ND, nondegradable; HFF, human foreskin fibroblast.
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Introduction

The most desirable anticancer therapy is both potent and specific toward tumor cells [1,2]. Many conventional small molecule chemotherapeutics do not discriminate between cancerous and normal cells, cause damage to healthy tissues, and are therefore unable to be administered at high dosage. In contrast, cytoplasmic and nuclear proteins that specifically alter the signaling pathways in tumor cells, reactivate apoptosis and restore tissue homeostasis, can delay tumor progression with less collateral damage to other tissues [3–6]. Using stimuli-responsive nanocarriers for the intracellular delivery of such proteins, including human tumor suppressors [7] and exogenous tumor-killing proteins [8–10]), is attractive as a new anti-cancer therapy modality.

Apoptin is a 121-residue protein derived from chicken anemia virus [9]. When transgenically expressed, apoptin can induce p53-independent apoptosis in a variety of tumor and transformed cells [11,12], while leaving normal and untransformed cells unaffected [13]. Apoptin exists as a globular multimeric complex, composed of thirty to forty subunits, with no well-defined secondary structure [14]. While the exact mechanism of the tumor selectivity is unresolved, apoptin is known to translocate to the nucleus where tumor-specific phosphorylation at residue Thr108 takes place, leading to accumulation of apoptin in nucleus and activation of the apoptotic cascade in tumor cell [15]. In normal cells, apoptin is not phosphorylated at Thr108 and is located mostly in the cytoplasm, where it aggregates and undergoes degradation [16]. Because of the high potency in inducing this exquisite tumor-selective apoptosis, apoptin has been investigated widely as an anti-tumor therapeutic option [9]. Different gene therapy approaches have been used to administer apoptin to mouse xenograft tumor models, in which significant reduction in tumor sizes and prolonged lifespan of mice have been observed without compromising the overall health [17–19]. However, as with other gain-of-function therapy candidates, in vivo gene delivery approaches using viral vectors may lead to unwanted genetic modifications and elicit safety concerns [20]. While protein transduction domain (PTD)-fused apoptin has been delivered to cells [21,22], this approach suffers from inefficient release of the cargo from endosomes and instability of the unprotected protein [23]. Development of nanoparticle carriers to aid the functional delivery of apoptin to tumor cells is therefore desirable [24].

We chose to work with recombinant maltose-binding-protein fused apoptin (MBP–APO) that can be solubly expressed from Escherichia coli, whereas native apoptin forms inclusion bodies [14]. MBP–APO has been shown to similarly assemble into a multimeric protein complex, which exhibits the essential functions and selectivity of native apoptin [14]. Nanoparticle-mediated delivery of functional MBP–APO poses unique challenges [25]. First, MBP–APO preassembles into large complex with an average diameter of ~40 nm and molecular weight of ~2.4 MDa [14]. To achieve nanocarrier sizes that are optimal for in vivo administration (~100 nm) [26], a loading strategy that forms compact particles is desirable. Second, in order to maintain the multimeric state of functional MBP–APO, the protein loading and releasing steps need to take place under very mild, physiological conditions in the absence of surfactants. Lastly, the nanocarrier must completely disassemble inside the cell to release the MBP–APO in its native and unobstructed form to ensure the correct spatial presentation of key residues within the apoptin portion, including the nuclear localization/export signals, the phosphorylation site and other elements important for downstream signaling.

In the current study, we selected a polymeric nanocapsule (NC) strategy for the functional delivery of MBP–APO, in which the protein complex is noncovalently protected in a water soluble polymer shell (Fig. 1). This slightly positively charged shell shields the MBP–APO from serum proteases and surrounding environment, while enabling cellular uptake of the polymer–protein complex through endocytosis [27]. The polymeric layer is weaved together by redox-responsive cross-linkers containing disulfide bond (S–S) that can be degraded once the NCs are exposed to the reducing environment in cytoplasm [28]. No covalent bonds are formed between the protein cargo and the polymer shell, which ensures complete disassembly of the capsule layer and release of native MBP–APO inside the cell. Using this approach, we show that MBP–APO can be efficiently delivered to induce apoptosis in cancer cell lines selectively both in vitro and in vivo.

Materials and methods

Materials

N-(3-aminopropyl)methacrylamide hydrochloride was purchased from Polymer Science, Inc. CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) reagent was purchased from Promega Corporation, APO-Brdu™ TUNEL Assay Kit was purchased from Invitrogen. In Situ Cell Death Detection Kit, POD; was purchased from Roche Applied Science. Female athymic nude (nu/nu) mice, 6 weeks of age, were purchased from Charles River Laboratories (Wilmington, MA). All other chemicals were purchased from Sigma–Aldrich and used as received.

Protein nanocapsule preparation

The concentration of protein was diluted to 1 mg/mL with 5 mM sodium bicarbonate buffer at pH 9. Then 200 mg/mL acrylamide (AAm) monomer was added to 1 mL of protein solution with stirring at 4 °C. After 10 min, the second monomer, N-(3-aminopropyl)methacrylamide (APMAAm), was added while stirring. Different cross-linkers, N,N'-methylene bisacrylamide for ND NC and N,N'-bis(acryloyl)cystamine for 5–5 NC, were added 5 min after the addition of APMAAm. The polymerization reaction was immediately initiated by adding 30 μL of ammonium persulfate (100 mg/mL, dissolved in deoxygenated and deionized water) and 3 μL of N,N,N',N'-tetramethylethylenediamine. The polymerization reaction was allowed to proceed for 60 min. The molar ratios of AAm/APMAAm/cross-linker used were
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Figure 1  Degradable nanocapsules for apoptosis delivery. (a and b) Schematic diagram of synthesis of degradable apoptosis nanocapsules (S-S APO NC) and delivery into tumor cells to induce apoptosis; TEM images of (c) native MBP—APO; (d) enlarged image of MBP—APO; (e) S-S APO NC; and (f) degraded S-S APO NC after treatment with 2 mM GSH for 6 h at 37 °C.
1.5:1:0.14, 2:1:0.14, 4:1:0.14, and 8:1:0.14. Buffer exchange with phosphate-buffered saline (PBS) buffer (pH 7.4) was used to remove the remaining monomers and initiators. Rhodamine-labeled APO NCs was obtained through encapsulation of MBP–APO modified with 5-carboxy-X-rhodamine N-succinimidyl ester (mass ratio (MBP–APO:rhodamine) = 4:1).

Characterization of protein nanocapsules

The mean hydrodynamic size and ζ-potential of NC were determined by dynamic light scattering (DLS) in PBS buffer. Samples of NSCs (0.05 mg/mL) for TEM imaging were negatively stained with 2% uranyl acetate in alcoholic solution (50% ethanol). The lamella of stained sample was prepared on carbon-coated electron microscopy grids (Ted Pella, Inc.).

Cellular uptake and localization of nanocapsules

MDA-MB-231, HeLa, MCF-7, and human foreskin fibroblast (HFF) cells (ATCC, Manassas, VA) were cultured in Dulbecco’s modified Eagle’s media (DMEM) (Invitrogen) supplemented with 10% bovine growth serum (HyClone, Logan, UT), 1.5 g/L sodium bicarbonate, 100 μg/mL streptomycin and 100 U/mL penicillin, at 37°C with 98% humidity and 5% CO₂. To visualize NCs uptake, MDA-MB-231 cells were seeded into 48-well plate, with a density of 10,000 cells/well in 250 μL of media with supplements. S–S Rho–APO NC and ND Rho–APO NC were added to a final concentration of 20 nM. After 1 h and 24 h of incubation, cells were washed with PBS twice, stained with DAPI Nucleic Acid Stain and imaged. For internalization of S–S Rho–APO NC with different ζ-potentials, MDA-MB-231 cells were incubated with 20 nM NCs for 2 h before nuclei staining. Markers for early and late endosomes were used for internalization trafficking study. A concentration of 20 nM S–S Rho–APO NCs was added to HeLa cells and incubated for 30 min, 60 min and 120 min under 37°C. Cells were then fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100, and stained with antibodies, mouse anti-EAA1 antibody against early endosomes and rabbit anti-CI-MPR antibody against late endosomes (Cell Signaling Technology, Inc.). Texas red goat anti-mouse IgG and Alexa Fluor® 647 goat anti-rabbit IgG (Invitrogen) were added as the secondary antibody. To determine the cellular localization of the protein delivered, confocal images were taken with HeLa, MCF-7, and HFF cells incubated with 20 nM of S–S Rho–APO NC or ND Rho–APO NC at 37°C for 24 h. Nuclei were then counterstained with DAPI. The Z-stack images of cells were imaged at 0.4-μm intervals and analyzed by Nikon NIS Element software. Fluorescent microscopy images were acquired on a Yokogawa spinning-disk confocal scanner system (Solamere Technology Group, Salt Lake City, UT) using a Nikon eclipse Ti-E microscope equipped with a 60×/1.49 Apo TIRF oil objective and a Cascade II: 512 EMCCD camera (Photometrics, Tucson, AZ, USA). An AOTF (acousto-optical tunable filter) controlled laser-merge system (Solamere Technology Group Inc.) was used to provide illumination power at each of the following laser lines: 491 nm, 561 nm, and 640 nm solid state lasers (50 mW for each laser).

Cytotoxicity assays

Different cancer cell lines, HeLa, MCF-7 and MDA-MB-231, as well as noncancerous HFF, were seeded into 96-well plates, each well containing 5000 cells in 100 μL of DMEM with supplements. Different concentrations of protein and NCs were added into each well and the plates. After incubation of 48 h at 37°C, the wells were washed with PBS solution twice and 100 μL of fresh cell culture media with supplements was added. Then 20 μL MTS solution (CellTiter 96® AQueous One Solution Cell Proliferation Assay) was added into each well and the plates were incubated for 3 h at 37°C. The absorbance of each well was read at 490 nm using a microplate reader (PowerWave X, Bio-tek Instruments, USA). Apoptosis was detected using APO-BrdU Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay kit. MDA-MB-231 and HFF cells were seeded at a density of 100,000 cells/well into a 6-well plate in 2 mL of cell culture media with supplements. Proteins and NCs were added after cells covered 80% of bottom surface. After 24 h of incubation, cells were fixed with 1% paraformaldehyde in PBS, followed by the addition of DNA labeling solution containing terminal deoxynucleotidyl transferase and bromodeoxyuridine (Brdu). Cells were then stained with Alexa Fluor® 488 dye-labeled anti-BrdU antibody. Samples were deposited onto slides, which were later stained with propidium iodide (PI) solution containing RNase A. Images were obtained by fluorescent microscope (Zeiss, Observer Z1) using appropriate filters for Alexa Fluor 488 and PI.

In vivo studies with MCF-7 xenograft model

All mice were housed in an animal facility at the University of Southern California in accordance with institute regulations. Female athymic nude (nu/nu) mice were subcutaneously grafted on the back flank with 5×10⁶ MCF-7 tumor cells. Afterwards, tumor size was monitored by a fine caliper and the tumor volume was calculated as the product of the two largest perpendicular diameters and the vertical thickness (L x W x D, mm³). When the tumor volume reached 100–200 mm³, mice were randomly separated into different groups. From day 0, mice were treated with intratumoral injection of native MBP–APO or S–S APO NC (200 μg/p mouse) every other day. PBS and S–S BSA NC were included as the negative controls. When the tumor volume exceeded 2500 mm³, the mice were euthanized by CO₂ according animal protocol. The average of tumor volumes was plotted as the tumor growth curve in respective treated groups. For histology study, treated tumor samples were collected and fixed in 4% paraformaldehyde, and processed for staining using the In Situ Cell Death Detection Kit. The stained tumor slides were observed under microscope, and representative pictures were taken for analysis. Paraformaldehyde-postfixed frozen tumor sections (5-μm thick) were permeabilized with 0.1% triton X-100 and stained with TUNEL assay kit (In Situ Cell Death Detection Kit, POD; Roche Applied Science, Indianapolis, IN) in accordance with
the manufacturer's instructions. DAPI was used for nuclear counterstaining.

Results and discussion

Synthesis and characterization of apoptin nanocapsules

MBP–APO (pl = 6.5) was first purified from E. coli extract using an amylose-affinity column (Supplement 2 and Supplement 8). Dynamic light scattering (DLS) measurement revealed an average hydrodynamic radius of 36.1 nm (Supplement 3), consistent with the reported size for the recombinant MBP–APO complex [14]. Transmission electron microscopy (TEM) analysis of MBP–APO showed similarly sized protein complexes (Fig. 1c and enlarged in Fig 1d). Interestingly, MBP–APO complexes appear to adopt a disk-shaped structure despite the lack of defined secondary structure from the apoptin component. Since the apoptin portion of the protein can self-assemble into the ~40-mer complex, we propose a three dimensional arrangement of MBP–APO in which the C-terminal apoptin forms the central spoke of the wheel-like structure (Fig. 1a), with the larger MBP portion distributes around the apoptin. The planar arrangement allows the apoptin portion of the fusion protein to remain accessible to its protein partners, which may explain how the MBP–APO fusion retains essentially all of the observed functions of native apoptin. The reversible encapsulation strategy for producing apoptin NCs is shown in Fig. 1a. Following electrostatic deposition of the monomers acrylamide (1 in Fig. 1a) and N-(3-aminopropyl)methacrylamide (2), and the cross-linker N,N'-bis(acryloyl)cystamine (3), at a molar ratio of 1:5:1:0.14, onto MBP–APO (1 mg) in carbonate buffer (5 mM, pH 9.0), in situ polymerization was initiated with the addition of free radical initiators and proceeded for 1 h. The molar ratio and the time of reaction reported were optimized to minimize protein aggregation and precipitation, as well as to maximize the solution stability of the resulting NCs (designated below as S–S APO NC). Excess monomers and cross-linkers were removed using ultrafiltration and S–S APO NC was stored in PBS buffer (pH 7.4). DLS clearly showed increase in average diameter of the sample to ~75 nm (Supplement 3) with a slightly positive ζ-potential value of 2.8 mV (Supplement 1). TEM analysis of the S–S APO NC confirmed the nearly doubling in diameter of the spherical particle (Fig. 1e). Unexpectedly, the NCs displayed dark contrast upon uranyl acetate staining, which hints that the cores of the particles were very densely packed. As expected from the incorporation of redox-responsive cross-linker 3, the reduction of NCs size can be seen upon treatment of the reducing agent glutathione (GSH) (2 mM, 6 h, 37°C). As shown in Fig. 1f, the densely packed NCs were completely dissociated into ~30 nm particles, confirming the reversible nature of the encapsulation process. As a control, we also synthesized nondegradable MBP–APO NCs (ND APO NC) using N,N'-methylene bisacrylamide as the cross-linker with same monomer and protein concentration under identical reaction conditions. Whereas similarly sized NCs were formed, no degradation of ND APO NC can be observed in the presence of GSH.

Cellular uptake and localization of nanocapsules

We next examined the cellular uptake of the S–S APO NC and cellular localization of the cargo. If the unique tumor selectivity of MBP–APO is maintained following the encapsulation and release processes, we expect the delivered MBP–APO to either accumulate in the nuclei of the tumor cells, or to localize in the cytoplasm of noncancerous cells. Prior to the polymerization process, the MBP–APO protein was conjugated to amine-reactive rhodamine (Rho–APO) as described in section ‘Protein nanocapsule preparation’. Subsequent encapsulation yielded similarly sized NCs as unlabeled S–S APO NCs. Fluorescent microscopy showed all NCs readily penetrated the cell membrane and are present in the cytoplasm of MDA-MB-231 cells within 1 h (Supplement 4). When the relative amounts of positively charged monomer 2 were reduced in the NC shell, corresponding decreases in ζ-potentials of the NCs were measured by DLS, which led to decreases in cellular internalization (Supplement 5). The cellular trafficking of the internalized S–S Rho–APO NCs in HeLa cells was investigated for 2 h by monitoring colocalization using fluorescent markers for early and late endosomes (Fig. 2a and Supplement 6). Colocalization of Rho–APO with early endosomes was detected at the highest levels after 30 min and decreased at later time points. In contrast, colocalization of Rho–APO with late endosome remained low throughout the trafficking studies. Colocalization of Rho–APO with nuclei became evident after 2 h, indicating endosomal escape and nuclear entry of the released apoptin protein. These results suggested that S–S Rho–APO NCs were trafficked into early endosomes upon internalization and at least a significant portion of the internalized NCs and the cargo can escape from the endosomal compartment.

To analyze protein localization using confocal microscopy, two cancer cell lines HeLa and MCF-7, together with the noncancerous human foreskin fibroblast (HFF), were treated with either S–S Rho–APO NC or ND Rho–APO NC (Fig. 2b). In the case of ND Rho–APO NCs, red fluorescence signals remained in the cytoplasm for all three cell lines, indicating the encapsulated Rho–APO proteins were well-shielded by the nondegradable polymer shell and the internal nuclear localization sequences were not accessible to the transport machinery. In stark contrast, when HeLa cells were treated with S–S Rho–APO NC, strong red fluorescence of rhodamine was present in the nuclei, resulting in intense pink color as a result of overlapping of rhodamine and DAPI fluorescence. Z-stacking analysis confirmed the Rho–APO to be localized inside of the nuclei. Similar results were observed with MCF-7 cells, although the fluorescence intensity was not as strong as in the HeLa cells. These results confirmed that the Rho–APO delivered can indeed be released in native forms inside the cytoplasm and enter the nuclei. More importantly, the tumor-specificity of delivered apoptin proteins toward cancer cell lines were demonstrated in the confocal analysis of noncancerous HFF cells treated with S–S Rho–APO NC, as all of the dye signals remained in the cytoplasm and no nuclear accumulation was observed.

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Tumor-selective cytotoxicity of apoptin nanocapsules

We then investigated whether the MBP–APO protein delivered still possesses its function to induce tumor-selective apoptosis. The potency and selectivity of S–S APO NC were tested on various cell lines including HeLa, MCF-7, MDA-MB-231, and HFF (Fig. 3a–d). MTS assay was used to measure cell viability 48h after addition of the protein and NC. For each cell line, ND APO NC and native MBP–APO were used as negative controls. When S–S APO NC was added to a final concentration of 200 nM, all three cancer cell lines had no viable cells, whereas ~75% of the HFF had survived. The IC\textsubscript{50} values were 80 and 30 nM for HeLa and MDA-MB-231, respectively. The IC\textsubscript{50} for MCF-7 was higher at ~110 nM, which may be due to the deficiency in the terminal executioner caspase 3 and reliance on other effector caspases for apoptosis [29,30]. As expected, native MBP–APO and ND APO NC did not significantly decrease the viability of any cell lines tested, consistent with the inability to enter cells and release MBP–APO in cytoplasm, respectively. The IC\textsubscript{50} values of S–S APO NC toward MDA-MB-231 increased as the surface charge of the NC became more neutral (Supplement 5), suggesting more efficient internalization can improve S–S NCs cytotoxicity. The morphologies of MDA-MB-231 and HFF cells were examined under various treatments. Only the S–S APO NC treated MDA-MB-231 cells exhibited blebbing and shrinkage, which are hallmarks of apoptotic cell death (Fig. 3e).
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Figure 3  Cytotoxicity and apoptosis observed following nanocapsule delivery. (a) HeLa; (b) MCF-7; (c) MDA-MB-231; or (d) HFF cells with treatment of different concentrations of S–S APO NC, ND APO NC, and native MBP–APO. (e) Apoptosis induced by S–S APO NC determined by TUNEL assay. Images on the left are bright field microscopy images of MDA-MB-231 and HFF cells treated for 24 h with 200 nM S–S APO NC. The scale bar represents 50 μm. Images right of the dash line shows detection of apoptotic fragmentation of the nucleosome after same treatment using APO-BrdU™ TUNEL assay kit. The scale bar represents 50 μm. Red fluorescence represents the propidium-iodide (PI)-stained total DNA, and green fluorescence represents the Alexa Fluor 488-stained nick end label, the indicator of apoptotic DNA fragmentation. The merged pictures combine the PI-stained nuclei and the Alexa Fluor 488-stained nick end label. (Note the bright field images do not overlap with the fluorescent microscopy images; cells were detached and collected for TUNEL assay after treatment.)

and Supplement 7). Using TUNEL assay, S–S APO NC treated MDA-MB-231 also showed nuclear fragmentation associated with apoptosis, whereas cells treated with native MBP–APO and ND APO NC at the same concentration (Supplement 7), as well as HFF treated with 200 nM S–S APO NC (Fig. 3e), had no sign of apoptosis. Collectively, these results demonstrated that the recombinant MBP–APO delivered by the degradable NCs retains the potency and selectivity as the transgenically expressed apoptosis in previous studies [9].

In vivo evaluation of apoptin nanocapsules

We further examined the potency of S–S APO NC in a mouse xenograft model. Female athymic nude (nu/nu) mice were subcutaneously grafted on the back flank with $5 \times 10^6$ MCF-7 breast cancer cells. When the tumor volume reached $100–200\, \text{mm}^3$ (day 0), mice were randomly separated into different groups and treated with intratumoral injection of PBS, MBP–APO, S–S APO NC. In addition, S–S NC with bovine serum albumin (S–S BSA NC) was added as a nonlethal...
Figure 4  Treatment of apoptin nanocapsules resulted in tumor growth retardation. (a) Significant tumor inhibition was observed in the mice treated by S–S APO NC. Female athymic nude mice were subcutaneously grafted with MCF-7 cells and treated with intratumoral injection of MBP–APO (n = 4) or S–S APO NC (n = 4) (200 μg/mouse) every other day. PBS (n = 3) and S–S BSA NC (n = 4) were included as negative controls. The average tumor volumes were plotted vs. time. Asterisks indicate injection days. (b) Detection of apoptosis in tumor tissues after treatment with different NCs. Cross-sections of MCF-7 tumors were stained with fluorescein-dUTP (green) for apoptosis and DAPI for nucleus (blue). The scale bars represent 50 μm.

protein cargo control to test the effects of the S–S NC polymer component on tumor cells in vivo. Tumors treated with saline, S–S BSA NC or native MBP–APO expanded rapidly and reached the maximum limit (>2500 mm³) within 12 days. In sharp contrast, tumor growth was significantly delayed when treated with S–S APO NC (Fig. 4a). Fixed tumor tissues collected from each treatment group were examined for DNA fragmentation using in situ TUNEL assay. The images revealed the highest level of cell apoptosis for the tumor harvested from mice treated with S–S APO NC, correlating well with the significantly delayed tumor growth observed for this treatment group and confirming that tumor growth inhibition was indeed due to apoptin-mediated apoptosis (Fig. 4b). Collectively, the xenograft study verified that the degradable NCs effectively delivered MBP–APO proteins to tumor cells in vivo, which was highly effective in limiting tumor progression. Upon further optimization of the pharmacokinetics of the S–S APO NC, including surface derivatization with active targeting ligands, these particles may be intravenously administered as an anticancer therapy [31].

Conclusions

We were able to deliver the high molecular weight complex of the tumor-selective MBP–APO using a redox-responsive polymeric NC in vitro and in vivo. The choice and design
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of the sub-100 nm NC is well-suited for diverse protein targets because of its mild preparation conditions, reversible encapsulation, efficient membrane penetration, and cytoplasmic release of the protein cargo. Our application here further illustrates how intracellular protein delivery using nanoscale system can provide new possibilities for achieving selective anticancer therapy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.nantod.2012.12.003.

References


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