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Engineering a customized nanodrug delivery system at the cellular level for targeted cancer therapy

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Abstract

Drug administration customized to individual cells could intrinsically address cancer heterogeneity and provide a safe and effective method for delivering personalized treatment. To accomplish this, we developed a smart nanodrug delivery system characterized by cancer cell-targeted drug delivery and intracellular biomarker-responsive drug activation. This system was composed of a long-nicked DNA duplex formed by tandem hybridization of two extended antisense oligonucleotides whose ends were separately blocked with a cancer cell-specific aptamer, AS1411, and a replaceable anti-biomarker probe (ABP). We demonstrated that this DNA nanodrug was directed to cancer cells with the guidance power of AS1411 and then activated by the presence of a given intracellular biomarker. By using such a belt-and-braces strategy, this DNA nanodrug system could safely and efficiently accelerate apoptosis of target cancer cells. Moreover, since the expression level of biomarkers tends to indicate the specific physiological state of individual cells, biomarker-responsive activation of the nanodrug is expected to enable customized drug administration at the cellular level.

Keywords

aptamer; drug delivery; personalized medicine; antisense oligonucleotides

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Conflict of interest The authors declare that they have no conflict of interest.

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1 Introduction

Cancer heterogeneity among patients with the same type of cancer, or even the same patient at different disease stages, can result in totally different responses to cancer medications [1]. Personalized medicine (PM), which uses individual genetic information as a guide toward providing “therapy with the right drug at the right dose in the right patient”, has attracted broad interest [2]. On the other hand, few studies have reported on cellular heterogeneity in cancer treatment research. Since no two cells are alike, even within the same cell type, medications customized to individual cells would provide optimal care under a regime of personalized care [3]. In particular, an ideal approach would utilize gene therapy, which can halt cancer progress with high specificity and potency through modulation of certain cancer genes [4]. However, to successfully apply gene therapy in PM at the cellular level, two primary requirements must be met: (1) efficient delivery of therapeutic nucleic acids to target cells; (2) precise drug activation according to the specific physiology of individual cells.

Cancer cell-targeted delivery of therapeutics enabling reduced systematic toxicity to healthy cells/tissues is essential for individual cell-based PM [5,6]. On the other hand, since nucleic acids are macromolecules with negative charge, they cannot freely pass through the plasma membrane. Thus, delivery systems are needed to facilitate their cellular penetration [7,8]. The advancement of DNA nanotechnology offers a vast number of multifunctional nanoscale platforms for transport of therapeutic nucleic acids with the additional merits of programmability, biocompatibility and biodegradability [9]. Moreover, by using a technique called cell-SE-LEX, a panel of functional oligonucleotides, namely aptamers, has already been selected for specific cell-surface phenotypes and used for cell-targeted delivery of molecular/nanosized cargo [10]. Although bench science has focused on aptamer-functionalized DNA nanoplateforms, transition to practical devices with simple engineering for clinical application is the ultimate goal.

To further reduce off-target effects and overcome cellular heterogeneity, it is necessary to precisely tailor the active dosage of delivered therapeutics according to the physiological demands of individual cells. To date, a wide spectrum of “on-demand” drug delivery systems has been designed by loading molecular drugs into stimuli-responsive nano-carriers, with drug release triggered by an exogenous stimulus, such as light [11], heat [12], magnetic field [13], ultrasound [14], or electric field [15]. Despite the ability to control spatiotemporal drug release, such systems are limited in their practical applications owing to complicated operation, requirements for sophisticated apparatus, and low *in vivo* manipulation efficiency [16]. Moreover, the released drug dosage is regulated by exogenous stimuli. Therefore, control over the process depends on the knowledge and experience of trained operators, not the physiological state of cells. In contrast, endogenous stimuli-responsive nano-carriers which dissolve, collapse, or swell in response to specific changes in the microenvironment, such as enhanced concentration of glutathione [17], increased temperature [18], lower pH [19], and higher level of reactive oxygen species [20], are more suitable to clinical settings. Although these disease-associated microenvironments provide some information relative to drug activation, they still do not reflect differences in physiology between individual cells. As measurable indicators of specific cellular states, biomarkers play an essential role in

identifying cellular heterogeneity. In addition, the programmable toehold-mediated strand displacement of nucleic acids has been used to develop logic-based DNA/RNA circuits for intelligent medicine [21]. With introduction of DNA logic-based platforms, it is possible to develop smart drug delivery systems, which could specifically sense a given biomarker and then customize cell-specific drug activation accordingly, as a positive step toward achieving PM at the cellular level.

As a proof-of-concept, we used DNA molecules as building blocks to construct this smart nanodrug (ND) delivery system for targeted personalized cancer therapy. A549, a human lung cancer cell line [22], was used as the model cell. As shown in Scheme 1, this nanostructure consists of three domains. The middle part, which serves as the therapeutic nanodrug, is a long-nicked DNA duplex formed by tandem hybridization of two DNA monomers, termed A and B, containing the antisense oligonucleotide sequence of two cancer-related genes, miR-21 and miR-150, respectively (Table S1). As reported, antisense inhibition of miR-21 [23] and miR-150 [24] can efficiently accelerate apoptosis of A549 cells, but can potentially induce completely opposite results on other cancer cell lines. For example, antisense inhibition of miR-21 could promote proliferation of HeLa cells [25]. Therefore, to guarantee the safety of antisense gene therapy, a strategy combining cancer cell-targeted delivery and customized activation of therapeutics was introduced through separately blocking the ends of ND with a cancer cell-specific aptamer AS1411 (Apt) and a replaceable anti-biomarker probe (ABP). The intact DNA nanoassembly was named as Apt-ND-ABP. It has been amply demonstrated that aptamer AS1411 can specifically recognize nucleolin, which is overexpressed on the cell membrane of many cancers, and, more importantly, it can shuttle between the cytoplasm and nucleus [26]. Based on the navigational specificity of AS1411, the functionalized nanodrug, Apt-ND-ABP, could be directed to the cytoplasm of A549 cells. A well-known proto-oncogene, c-raf-1 mRNA, whose expression level is intrinsically associated with the growth and development of A549 cells [27], was chosen as a gene bio-marker. More specifically, high expression level of c-raf-1 mRNA reflects A549 cells in a state of severe tumorigenesis, while cells expressing low c-raf-1 mRNA presumptively represent normal cells or non-A549 cancer cells. In the absence of c-raf-1 mRNA, the nanodrug remains inert with its two ends blocked. However, upon sensing c-raf-1 mRNA, ABP is removed through the formation of a c-raf-1 mRNA/ABP complex, resulting in activation of the nanodrug by exposure of the nanodrug toehold. As such, multiple anti-sense oligonucleotides, A and B monomers, can access and then inhibit therapeutic target miRNAs, miR-21 and miR-150, respectively, thus leading to acceleration of A549 cell apoptosis. Since activation of the nanodrug depends on the expression level of c-raf-1 mRNA, which indicates the specific cancerous state of individual cells, this system is expected to achieve customized drug administration for personalized cancer gene therapy at the cellular level.

2 Experimental

2.1 Fabrication of the nanodrug

Oligonucleotides designed in this study were synthesized by Sangon Biotech (Shanghai, China). All oligonucleotide products were first dissolved in sterile water with a stocking

concentration of 100 μM . The fabrication of the nanodrug was conducted as follows. First, monomer A (5 μL), monomer B (6 μL) (the DNA sequences see the Supporting Information online) and Dulbecco's phosphate buffered saline (DPBS) supplemented with 25 mM MgCl_2 (4 μL) were mixed in tube 1. Monomer C (6 μL), ABP (6 μL) and DPBS supplemented with 25 mM MgCl_2 (4 μL) were mixed in tube 2. Apt (6 μL) and DPBS supplemented with 25 mM MgCl_2 (2 μL) were mixed in tube 3. To promote the tandem self-assembly of A and B, tube 1 was placed in boiling water and then cooled down slowly in a sealed foam box for 24 h, and the resulting products were used as the ND. To functionalize ND with a biomarker recognition probe and a cell-targeted ligand, the solutions in tube 2 and tube 3 were added into tube 1, and the mixture was allowed to incubate at room temperature for 12 h. In this way, the ends of ND could be separately blocked with excess Apt and C-ABP moiety through hybridization. Finally, the resultant product, Apt-ND-ABP, was purified through Amicon Ultra Centrifugal Filter (100 kD, Millipore, USA) to remove excess oligonucleotides and the relatively short nanodrugs.

2.2 Atomic force microscopy imaging

To prepare the sample for atomic force microscopy imaging, 20 μL of Apt-ND-ABP (4 nM) was mixed with 4 μL of Ni^{2+} (300 μM). The mixture was slowly deposited on mica and dried at room temperature for 1 h. After rinsing with ultra-pure water four times and drying again, the sample was imaged on a Multimode 8 (Bruker, USA) using ScanAsyst mode and analyzed with the Nanoscope analysis software.

2.3 Agarose gel electrophoresis

To prepare a 1.5% agarose gel, 0.45 g agarose and 1 μL ethidium bromide were mixed into 30 mL 1 $\times$ Tris-borate-EDTA (TBE) buffer, followed by heating and cooling steps. Each sample (10 μL) was mixed with 2 μL 6 $\times$ loading buffer before loading in the gel. The electrophoresis experiments were conducted at 110 V on ice for 45 min. The resultant gels were imaged using a Bio-Rad molecular imager under UV light.

2.4 Western blot analysis

A549 cells were grown on 35-mm culture plates until about 70% confluence before experiments. After being washed with DPBS three times, the cells were incubated with Apt-ND-ABP (500 nM) for 6 h. Then, the culture medium was replaced with 1 mL fresh culture medium plus 10% fetal bovine serum (FBS, Gibco, Suzhou, China). After another 48-h incubation, the cells were washed with DPBS three times and lysed with 50- μL cell lysis solution. The resulting proteins were collected by centrifuge at 14000 $\times g$ for 20 min, and the protein concentrations were determined with a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). Proteins were separated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 110 V for 90 min and electrotransferred to nitrocellulose membranes at 300 mA for 90 min. The membranes were blocked with 5% nonfat dry milk for 1 h and then incubated with specific primary antibody overnight. After washing with TBST (a kind of buffer solution containing 20 mM Tris-HCl, 137 mM NaCl and 0.1% Tween20) three times, the membranes were incubated with secondary antibody for 1 h. After being washed with TBST and treated with imaging reagents, protein expressions were measured using a Bio-Rad molecular imager with imaging software.

2.5 Cell lines and cell culture

All cells used in this experiment were purchased from American Type Culture Collection. All cell lines were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% FBS (heat-inactivated) and 100 IU/mL penicillin-streptomycin (Cellgro, China).

2.6 Flow cytometry assay

To perform the binding assay using flow cytometry, both aptamer and library sequence were labelled with a fluorescein isothiocyanate (FITC) fluorophore. First, cells were treated with 0.2% EDTA and then collected through centrifugation (800 r/min, 5 min). After washing and counting, 2×10^5 cells were incubated with free aptamer, library sequence, Apt-ND-ABP or Lib-ND-ABP in 500 μ L binding buffer (prepared by adding 1 g albumin from bovine serum (BSA), 100 mg tRNA, 4.5 g of glucose and 5 mL of 1 M MgCl_2 to 1 L of DPBS) on ice for 30 min. The concentrations of aptamer, library sequence, Apt-ND-ABP and Lib-ND-ABP were fixed at 250 nM. After washing three times with washing buffer (prepared by adding 4.5 g of glucose and 5 mL of 1 M MgCl_2 to 1 L of DPBS), the cells were suspended in DPBS (400 μ L) and analyzed on a BD FACSVerse™ flow cytometer. The data were analyzed with FlowJo software.

2.7 Confocal fluorescence microscopy imaging

Cells (10^5) were plated in a 35-mm confocal dish and grown at 37 °C with 5% CO_2 for 24 h. After washing three times with DPBS, the cells were incubated with fluorescent aptamer or fluorescent nanodrug at 37 °C for the required time. After that, the cells were washed three times with washing buffer. Then 200 μ L FBS-free DMEM medium were added to each dish, and the cells were imaged using a FV1000 confocal microscope (Olympus, Japan). The data were analyzed with FV10-ASW 2.0 Viewer software.

2.8 MTS assay

Cells (3000) were grown in a 96-well plate for 24 h. After removal of the medium, the cells were incubated with Apt-ND-ABP, Lib-ND-ABP, Apt-ND, Apt-ND-rd, or Apt-ctrl-ABP at 37 °C with 5% CO_2 for 4 h. Then supernatant was removed, followed by adding 200 μ L fresh culture medium (10% FBS). The cells were further incubated at 37 °C with 5% CO_2 for 48 h. After removal of the medium, the cells were washed with DPBS and then incubated with 100 μ L MTS (3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) reagent at 37 °C for 1 h. Finally, the 450-nm absorption of the resulting cell samples was measured using a microplate reader.

3 Results and discussion

3.1 Formation and characterization of ND

ND formation was demonstrated using agarose gel electrophoresis (Figure S1, Supporting Information online) and atomic force microscopy (Figure 1(a)), and the patterns were consistent with our previous work [28]. To avoid “uncapped” events, excess Apt and ABP were added to block the free ends of ND. The resultant Apt-ND-ABP was purified and collected by an Amicon Ultra Centrifugal Filter, and the average length was ~250 nm,

equalling ~18 A/B duplex units. Such a high payload of antisense therapeutic agents is compatible with, or superior to, that of many nanocarriers [29]. Moreover, the antisense oligonucleotides themselves formed the nanostructure in this design, avoiding introduction of toxic and nonbiodegradable materials. The biomarker-responsive feasibility of this nanodrug system was first evaluated in buffer solution using DNA analogs of miR-21, miR-150 and c-raf-1 mRNA (termed as TA, TB, and BM, respectively) using agarose gel electrophoresis. As shown in Figure 1(b), without addition of BM, only slight disassembly of Apt-ND-ABP complexes was induced by TA and TB (Lane 2). However, after introduction of BM, a large number of Apt-ND-ABP complexes were dissociated to form the A/TA and B/TB duplexes (Lane 3), demonstrating the BM-initiated disassembly of Apt-ND-ABP. To demonstrate the sequence specificity of ABP upon nanodrug dissociation, the biomarker recognition toehold of ABP was replaced with a random sequence, and the corresponding nanocomplex (termed Apt-ND-rd) was tested. No detectable dissociation of Apt-ND-rd occurred on addition of TA, TB and BM (Lane 4 and Lane 5), revealing that the specific interaction between ABP and BM is a key point for activating the nanodrug. The biomarker-specific activation of Apt-ND-ABP was further confirmed by a much weaker dissociation (no more than 23%) induced by a single-base mutant of BM (Figure S2).

3.2 Cancer cell-specific delivery

Having confirmed the biomarker-responsive feasibility of Apt-ND-ABP in buffer solution, we next investigated its performance in cells. The higher expression level of c-raf-1 mRNA in cancerous A549 cells, compared with that in a normal cell line (WPMY-1), was first verified through fluorescence *in situ* hybridization assay (FISH, Figure S3). To evaluate the cell-specific binding affinity of Apt-ND-ABP, the aptamer sequence was labelled with FITC fluorophore. Figure 2(a) shows a significant FITC fluorescence shift of A549 cells incubated with Apt-ND-ABP, while only negligible fluorescence was observed from the cells incubated with Lib-ND-ABP, in which the aptamer sequence was replaced with a library sequence. Of note, the fluorescence signal of A549 cells incubated with free FITC-labelled aptamer was similar to that incubated with Apt-ND-ABP, indicating that incorporation of the DNA nanostructure has little impact on the specific binding affinity of the aptamer. However, no fluorescence shifts were observed in HBE, a normal human bronchial epithelial cell line, incubated with either free aptamer or Apt-ND-ABP (Figure 2(b)), revealing the selective recognition of both aptamer and Apt-ND-ABP to target A549 cells. Meanwhile, the specific binding of both aptamer and Apt-ND-ABP to other cancer cell lines (DU145 and MCF-7), but not normal cell lines (WPMY-1 and GES-1), was also demonstrated (Figure S4), suggesting the potent cancer-specific recognition capability of aptamer AS1411. Cellular uptake and intracellular distribution of Apt-ND-ABP were further studied with confocal laser scanning microscopy (CLSM) measurements. As shown in Figure 2(c), an obvious FITC fluorescence signal was observed in A549 cells incubated with Apt-ND-ABP, while that from Lib-ND-ABP-treated A549 cells, as well as that from free aptamer-/Apt-ND-ABP-treated HBE cells (Figure 2(d)), were much weaker, verifying the aptamer-guided cancer cell-specific internalization of the nanodrug. In addition, as demonstrated with lysotracker colocalization staining assay, a major amount of Apt-ND-ABP was localized out of lysosome, which could be explained by the non-endocytic internalization pathway of

aptamer AS1411 [30]. The escape of the nanodrug from the lysosomal compartment is important for implementing anti-miRNA gene therapy (Figure S5).

3.3 Biomarker-responsive activation of Apt-ND-ABP

To evaluate the biomarker-responsive activation of the nanodrug in cells, the middle of A was modified with a Cy3 fluorophore, and the 3' end of B was modified with a Cy5 fluorophore (Table S1, Supporting Information online). Within the A/B nanoassembly structure, the Cy3 and Cy5 fluorophores are kept in close proximity, resulting in a strong Cy3-Cy5 FRET signal. In contrast, the dissociation of the nanodrug separates these two fluorophores, leading to a decrease of FRET signal. In this experiment, A549 cells were incubated with the Cy3/Cy5-labelled nanodrug (Apt-ND-ABP or Apt-ND-rd) at 37 °C for 2 h and then imaged with CLSM. As shown in Figure 3(a), the cells treated with Apt-ND-ABP displayed an apparent Cy3 fluorescence signal, but a negligible FRET signal, indicating a remarkable disassembly of Apt-ND-ABP. However, in the cells treated with Apt-ND-rd, the Cy3 fluorescence signal was relatively weaker, accompanied by a strong FRET signal, revealing that most of the Apt-ND-rd assembly remained intact. The ratio of the Cy5 FRET signal to the Cy3 signal (termed as the FRET ratio) was calculated to assess the assembly/disassembly of the nanostructure. As shown in Figure 3(c), the FRET ratio induced by Apt-ND-ABP was ~6 times lower than that induced by Apt-ND-rd, indicating a higher disassembly of Apt-ND-ABP in comparison to Apt-ND-rd. To further confirm the biomarker-responsive activation of Apt-ND-ABP, the intracellular amount of the biomarker sequence was artificially increased and decreased by transfecting BM and its cDNA sequence (anti-BM) into A549 cells, respectively. The disassembly of Apt-ND-ABP was enhanced when increasing the intracellular amount of BM, but decreased with addition of anti-BM (Figure 3(d) and Figure S6), demonstrating that the activation of Apt-ND-ABP is dependent on the expression level of the biomarker. Next, the kinetic conformational changes of the nanodrug were investigated by incubating A549 cells with Apt-ND-ABP or Apt-ND-rd at 37 °C for 2 h. Then the cells were washed to remove free nanodrug and further incubated at 37 °C for different spans of time. The cells treated with Apt-ND-rd showed an apparent FRET signal with little signal decay, even after 36-h incubation (Figure S7). However, for cells treated with Apt-ND-ABP, the FRET signal decreased rapidly, and only a slight FRET signal could be observed after 1-h incubation. All these live-cell imaging results demonstrated that the specific biomarker-ABP interaction could induce a toehold-mediated strand displacement, thus promoting disassembly of the DNA nanodrug.

3.4 Biomarker-responsive and cancer-specific cytotoxicity of Apt-ND-ABP

After verifying the cancer cell-targeted delivery and bio-marker-responsive activation of the nanodrug system, we proceeded to test its therapeutic efficacy using an MTS assay. As shown in Figure 4, treatment of A549 cells with Apt-ND-ABP led to a remarkable dose-dependent inhibition of cell proliferation with an IC_{50} of 1 μ M. This therapeutic efficacy is comparable to that induced by the always-active nanodrug, Apt-ND, which has the same design as Apt-ND-ABP, but without ABP blockage. These results indicated that the biomarker recognition process had no negative impact on the therapeutic effect of Apt-ND-ABP. In contrast, without biomarker initiation, Apt-ND-rd remained in a quiescent state, resulting in negligible cytotoxicity, thus verifying the important role of the biomarker-

responsive activation of the nanodrug in therapeutic efficacy. We then demonstrated that the therapeutic effect of Apt-ND-ABP mainly originated from the antisense inhibition of target miRNAs. To accomplish this, a control system, termed as Apt-ctrl-ABP, was employed. Here, the middle domain was formed by tandem hybridization of two control sequences, neither of which matched the human genome RNA. No more than 15% inactivity was observed at the highest concentration (2 μ M of A/B monomer), indicating relatively low contributions of Apt and ABP to the therapeutic efficacy of Apt-ND-ABP on A549 cells. Meanwhile, as demonstrated with Western blot, the related proteins of miR-21 (PTEN) and miR-150 (SRCIN 1) were upregulated after treatment with Apt-ND-ABP (Figure S8). These observations were consistent with previous reports [31,32], further verifying the anti-miRNA therapeutic effect of Apt-ND-ABP. In addition, the selective therapeutic effect of our nanodrug system was confirmed by the much lower cytotoxicity of Lib-ND-ABP on A549 cell activity, as well as much lower cytotoxicity of Apt-ND-ABP on normal HBE cells (Figure S9), in comparison to that of Apt-ND-ABP on A549 cells. Collectively, these results demonstrated that the current biomarker-responsive nanodrug delivery system (Apt-ND-ABP) can provide customized drug administration to efficiently induce apoptosis of individual target cancer cells.

4 Conclusions

In summary, the aim of personalized cancer therapy at the level of individual cells was realized by the “belt-and-braces” policy of cancer-specific delivery and biomarker-responsive activation built into a smart nanodrug system. This system consisted of three domains: an aptamer for cancer-targeted delivery, a long DNA assembly formed by tandem hybridization of two therapeutic antisense oligonucleotides, and a replaceable probe for biomarker sensing. Activation of the nanodrug was dependent on the expression level of the biomarker, in this case, c-raf-1 mRNA, which indicates the specific cancerous state of individual cells, thus enabling precise tailoring of drug dosage at the cellular level. In addition, this system possessed several unique features which made it a desired platform for cancer research. (1) Aptamer AS1411 allowed cancer cell-specific internalization of the nanodrug in a nonendocytic pathway, which, to some degree, protected the DNA nanostructure from nuclease digestion. (2) This nanodrug delivery system was composed of pure nucleic acids, thus avoiding the involvement of toxic and nonbiodegradable nanocarriers and the concomitant accumulation and toxicity of other materials. (3) This universal gene delivery system could be easily extended to other cancer treatments by changing the targeted, therapeutic and/or biomarker-responsive modules. Finally, this gene delivery system for customized drug administration provides a new direction for the development of smart therapeutic strategies in personalized medicine. Still, owing to the complexity and heterogeneity of cancer, much effort is needed in the areas of discovery, identification, and rational selection of specific cancer biomarkers to turn this proof-of-concept system into clinical practicality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(a) Formation of nanodrugs characterized by atomic force microscope (AFM); (b) biomarker-responsive dissociation of the Apt-ND-ABP confirmed with 1.5% agarose gel electrophoresis. From Lanes 1 to 5: Apt-ND-ABP; Apt-ND-ABP+TA+TB; Apt-ND-ABP+TA+TB+BM; Apt-ND-rd; Apt-ND-rd+TA+TB+BM. Each sample was incubated at 37 °C for 1 h and then analyzed by agarose gel electrophoresis. The concentrations of A, B, TA, TB and BM were all fixed at 1 μ M (color online).

Figure 2.

Cell-specific binding and internalization of Apt-ND-ABP. Flow cytometry confirms the specific binding of Apt-ND-ABP to target A549 cells (a), but not control HBE cells (b). (c) CLSM images of A549 cells incubated with Apt-ND-ABP (i) and Lib-ND-ABP (ii) at 37 °C for 2 h. (d) CLSM images of HBE cells incubated with Apt (i) and Apt-ND-ABP (ii) at 37 °C for 2 h. The excitation wavelength of the FITC fluorophore was 488 nm (color online).

Figure 3.

Biomarker-responsive feasibility of Apt-ND-ABP in cells. CLSM images of A549 cells incubated with Apt-ND-ABP (a) and Apt-ND-rd (b) at 37 °C for 2 h. From left to right: fluorescence image of Cy3, FRET fluorescence image of Cy5, and bright field. The 543-nm laser was used for excitation. (c) FRET ratio calculated with ImageJ software accordingly. (d) FRET ratio calculated from the CLSM images of the A549 cells separately transfected with different amounts of BM (black line) and anti-BM (red line) prior to incubation with Apt-ND-ABP (color online).

Figure 4. Viability of A549 cells treated with Apt-ND-rd, Apt-ND-ABP, Lib-ND-ABP, Apt-control-ABP, and Apt-ND, respectively. Error bars represent the standard deviation of three independent experiments (color online).

Scheme 1.

Schematic illustration of the biomarker-responsive nanodrug delivery system for targeted cancer therapy at the cellular level. Apt-ND-ABP is composed of the nanodrug with two ends separately blocked with aptamer AS1411 and Anti-biomarker probe ABP. Apt-ND-ABP passes through the cell membrane guided by AS1411. In the absence of biomarker/ABP interaction, the nanodrug is quiescent. However, upon sensing the biomarker as an indicator of the specific physiological state of individual cells, ABP is removed by formation of a biomarker/ABP complex, followed by exposure of the nanodrug toehold for access to therapeutic target miRNAs (miRNA-21 and miRNA-150) for cell apoptosis (color online).