Drug Delivery

DOI: 10.1002/anie.200901452

Reversible Cell-Specific Drug Delivery with Aptamer-Functionalized Liposomes**

Zehui Cao, Rong Tong, Abhijit Mishra, Weichen Xu, Gerard C. L. Wong, Jianjun Cheng,* and Yi Lu**

cis-Diamminedichloroplatinum(II) (cisplatin) is a potent chemotherapeutic agent for the treatment of a broad range of cancerous tumors.[1,2] Despite its excellent antitumor efficacy, the major drawbacks of cisplatin include its lack of tumor specificity and severe side effects.[3] In addition, certain tumor-cell types develop resistance to cisplatin from exposure to the drug.[1] Strategies that allow the delivery of cisplatin specifically to tumor cells are highly desirable. Several strategies have been reported for the delivery of cisplatin specifically to tumor sites,[4–8] the most common of which is to use antibody (Ab) recognition against different cell-surface targets.[6–8] The binding of Abs to the cell-membrane receptors triggers receptor-mediated endocytosis, with the result of improved therapeutic efficacy.[9] Despite this success, the use of Abs as cell-specific homing agents poses significant challenges. Ab conjugations are difficult to control and typically show poor site specificity for the conjugation and inconsistent binding affinity.[9] The antibody-based drug-delivery system also tends to be immunogenic,[10] so it requires extra humanization steps, which make it more difficult for clinical application.

Nucleic acid based aptamers provide excellent alternatives to antibodies as cell-specific agents. They are single-stranded oligonucleotides identified through an in vitro selection process, termed system evolution of ligands by exponential enrichment (SELEX), to bind the target molecules selectively.[11,12] Many aptamers identified by SELEX have nearly identical binding affinity and specificity to those of Abs. Aptamers are much easier to prepare and to scale up. They are generally considered nonimmunogenic and can be gradually degraded by nucleases and cleared from the blood to cause minimal system toxicity. Functionalizations of aptamers to facilitate site-specific conjugation are also straightforward. Thus, aptamers are promising targeting ligands[13–24] and have been used in targeted drug-delivery systems, most of which are block-copolymer nanoparticles.[25–29] Although these new nanotechnology-based platforms look promising, the clinical benefit of nanoparticles for targeted cancer therapy is yet to be demonstrated.

Liposomes are by far the most successful drug-delivery systems;[30] a number of liposome-based systems have been approved by the US Food and Drug Administration for disease treatment in the clinic.[31] Liposomes have been shown to increase the plasma residence time of aptamers.[32] Previous efforts on liposomal drug delivery have focused on developing long-circulating liposomes that target cancerous tumor tissues through the enhanced permeation and retention (EPR) effect,[33,34] a passive targeting mechanism. However, cancer targeting entirely based on EPR still has undesirable systemic side effects and suboptimal antitumor efficacy:[35,36] clinical studies of a cisplatin-containing liposome showed only poor to moderate therapeutic efficacy.[37,38] Delivery vehicles with active tumor-targeting capability could, in principle, improve this significantly.[39]

Personalized chemotherapy is an unmet challenge in cancer treatment. Despite the existence of rough empirical dosage guidelines, the individual patient response can deviate strongly from average behavior. This problem is especially acute for chemotherapy agents, for which drug overdosage can have severe consequences. At present, once an initial dosage is administered, the side effects and drug effectiveness can no longer be modulated if there are no "antidotes" to the treatment. However, the discovery of good antidotes or neutralizers for each individual drug molecule is not an easy task, if even practical. Moreover, there are no known ways to "multiplex" different antidotes to control complex treatment profiles with multiple drugs.

We report here the controlled formulation of aptamer-conjugated, cisplatin-encapsulating multifunctional liposomes. Cancer-cell-specific targeting and drug delivery are demonstrated by using this delivery platform. Furthermore, we also show for the first time that a complementary DNA (cDNA) of the aptamer can function as an antidote to disrupt

[*] R. Tong,[+] A. Mishra, Prof. G. C. L. Wong, Prof. J. Cheng, Prof. Y. Lu
Department of Materials Science and Engineering
University of Illinois at Urbana-Champaign
1304 W. Green Street, Urbana, IL 61801 (USA)
Fax: (+1) 217-333-2736
E-mail: gclwong@illinois.edu
jianjunc@illinois.edu
Homepage:
http://www.matse.illinois.edu/faculty/wong/profile.html
http://www.matse.illinois.edu/faculty/cheng/profile.html
Dr. Z. Cao,[+] W. Xu, Prof. Y. Lu
Department of Chemistry
University of Illinois at Urbana-Champaign
600 S. Mathews Avenue, Urbana, IL 61801 (USA)
Fax: (+1) 217-244-3186
E-mail: yi-lu@illinois.eduyi-lu@illinois.edu
Homepage: http://www.chemistry.illinois.edu/faculty/Yi_Lu.html
[+] Z.C. and R.T. contributed equally to this work.

[**] This work was supported by a National Science Foundation Nanoscale Science and Engineering Center grant (DMR-0117792). J.C. thanks support from the National Science Foundation Career Program (DMR-0748834) and the Siteman Center for Cancer Nanotechnology Excellence (SCCNE, Washington University)—Center for Nanoscale Science and Technology (CNST, UIUC).
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200901452.

aptamer-mediated targeted drug delivery. This strategy for reversible delivery can, in principle, be adapted to a broad range of chemotherapy agents.

To demonstrate the effectiveness of our approach, we use an aptamer derived from AS1411, a 26-mer DNA aptamer that was recently discovered to have high binding affinity to nucleolin (NCL),[40] a *bcl-2* mRNA binding protein involved in cell proliferation.[41] NCL overexpression on the plasma membrane has been linked to various human diseases such as breast cancer,[41–44] therefore it provides a potential target for cancer-cell-specific drug delivery. AS1411 has been shown to exhibit antiproliferation activity in several cancer cell lines, including breast cancer cells (for example, MCF-7 and MDA-MB-231 cells[45]), presumably through its binding to NCL.[46] Thus, AS1411 is a promising targeting ligand for breast cancer. The sequence of the aptamer used in this study (called the NCL-aptamer) is 5′-GGT GGT GGT GGT TGT GGT GGT GGT GGT TTT TTT TTT TT-cholesterol-3′. The cholesterol tag immobilizes the aptamer on the liposome surface by inserting into the hydrophobic lipid membrane. The 12 extra T bases at the 3′-terminus ensure that the binding domains of the aptamer extend away from liposome surface, because the aptamer's capability of binding to the target may otherwise be compromised. To confirm the internalization of the aptamer by the MCF-7 cells, we labeled the AS1411 aptamer, as well as a control DNA of a scrambled sequence, with a 5′-end TAMRA dye, and then incubated them separately with MCF-7 cells at 37 °C for 10 h. The NCL-aptamer-treated cells showed much stronger TAMRA fluorescence in the cytoplasm than the control-DNA-treated cells (data not shown) when they were analyzed with a confocal microscope. The fluorescence was observed predominantly in the cytoplasm instead of in the nucleus of the MCF-7 cells, which was consistent with the projected endocytosis-based internalization mechanism.

The study mentioned above suggests that the NCL-aptamer may facilitate the endocytosis of drug-delivery vehicles. We next prepared NCL-aptamer-functionalized liposomes (NCL-aptamer–liposomes) of approximately 200 nm in diameter. The parental liposomes were formulated by following published procedures,[7,47,48] and were composed of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and distearoyl phosphatidylethanolamine derivatized with methoxy poly(ethylene glycol) with a molecular weight (M_W) of 2000 Da (mPEG2000–DSPE) at a molar ratio of 2/1/0.16. Such a composition renders liposomes with high rigidity and low permeability at 37 °C because of the relatively high transition temperature of HSPC.[49] The inert, hydrophilic PEG is commonly used in the formulation of liposomes for reduced nonspecific uptake.

The NCL-aptamer–liposome was prepared by mixing the above-mentioned reagents with the cholesterol-tagged NCL-aptamer during the hydration step of the liposome formulation. To monitor the internalization of the liposomes, we encapsulated calcein, a hydrophilic dye that has been frequently used for tracing cell fusion and division because of its impermeability to cell membranes. MCF-7 cells (NCL(+)) and LNCaP cells (NCL(−)) were incubated separately with calcein-containing NCL-aptamer–liposomes for 5 h, washed with phosphate-buffered saline (PBS), and then fixed with formaldehyde before being examined with a confocal microscope. Strong fluorescence was observed in the MCF-7 cells treated with NCL-aptamer–liposomes, whereas the fluorescence of the LNCaP cells treated with the NCL-aptamer–liposomes was noticeably much weaker (Figure 1). This study demonstrated that the binding capability of the

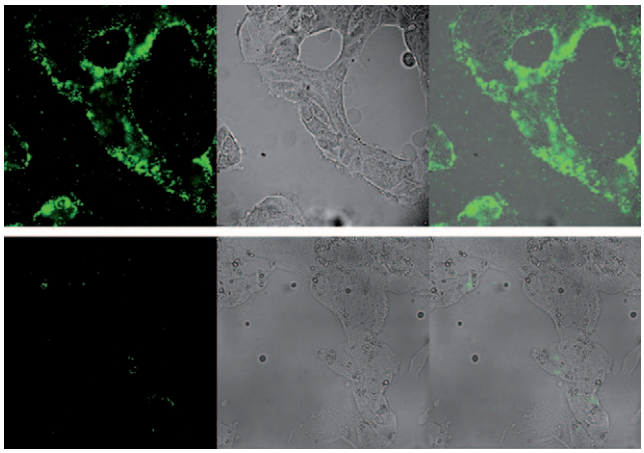

Figure 1. Confocal images of MCF-7 (top) and LNCaP (bottom) cells treated with NCL-aptamer-functionalized liposomes containing calcein. From left to right: fluorescence image, transmission image, and overlay.

NCL-aptamer was well preserved during liposome formulation and that the liposomes could be internalized into cells through NCL-mediated endocytosis. The latter observation was further confirmed by an uptake study evaluated by a fluorescence-activated cell sorting (FACS; Figure 1 in the Supporting Information).

We investigated the antiproliferation activity of the aptamer-conjugated liposomes by changing the encapsulated dye to the chemotherapy drug cisplatin. The viability of cells treated with NCL-aptamer–liposome–cisplatin was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against three control groups (Figure 2). Besides MCF-7 cells treated with aptamer–liposome–cisplatin, we also tested MCF-7 cells treated with aptamer–liposomes without cisplatin, MCF-7 cells treated with random-DNA-modified liposomes containing cisplatin, and LNCaP cells treated with aptamer–liposome–cisplatin. Figure 2 shows the viability results from these tests. About 21.5 % at day 2 and approximately 59.5 % at day 4 of the MCF-7 cells treated with aptamer–liposome–cisplatin displayed cell death. The two control tests involving cisplatin still had cell viability of about 88.6 % and 88.9 % at day 4, which suggests liposomes based on the control-DNA non-NCL-aptamer had no significant effect on cell viability. MCF-7 cells treated with aptamer–liposomes containing no cisplatin showed no noticeable cell death, with a viability of about 97.8 % even at day 4. Even though the AS1411 aptamer was reported to have cytotoxicity towards MCF-7 cells,[45] the quantity of aptamers used in this work was obviously not enough to cause any cell death. It is important to note that it is

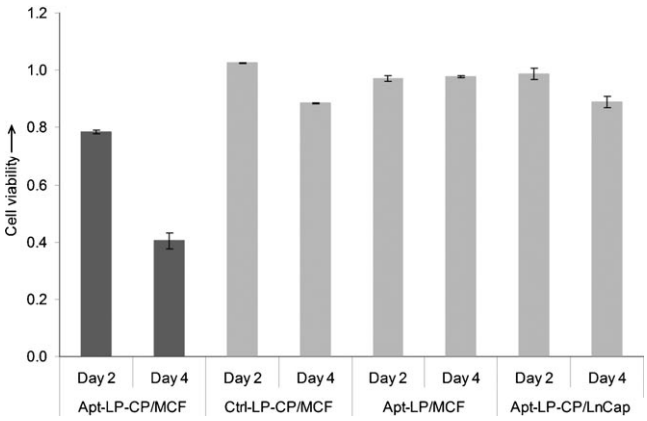

Figure 2. Cell viability assays for different liposome/cell combinations. Viability measured at day 2 and day 4 of the liposome/cell incubation. Columns 1 and 2: MCF-7 cells treated with NCL-aptamer-functionalized liposomes loaded with cisplatin (aptamer–liposome–cisplatin or Apt-LP-CP); columns 3 and 4: MCF-7 cells treated with a control (Ctrl) group of DNA-functionalized liposomes loaded with cisplatin (Ctrl-LP-CP); columns 5 and 6: MCF-7 cells treated with NCL-aptamer-functionalized liposomes with no cisplatin (Apt-LP); columns 7 and 8: LNCaP cells treated with NCL-aptamer-functionalized liposomes loaded with cisplatin (Apt-LP-CP).

much more economical to use aptamers as cell-specific targeting agents rather than therapeutic agents because the quantity needed is much lower and aptamers are usually more costly than small-molecule drugs.

The cytotoxicity of cisplatin to MCF-7 and LNCaP cells has been previously evaluated, with the IC_{50} values being 28 μM and 5.95 μM, respectively.[50,51] These numbers indicate that LNCaP cells are more prone to cisplatin-induced cell death than MCF-7 cells. However, by confining the cisplatin in liposomal vesicles and functionalizing the liposomes with cell-specific aptamers, much greater cell damage was observed for MCF-7 cells than for LNCaP cells. This comparison further supports the enhanced, receptor-mediated cell uptake and improved therapeutic efficacy of liposomes containing an aptamer targeting ligand.

Even though cancer-specific treatment helps reduce side effects, it is uncommon that a cancer-related receptor only exists on one type of cells and not any other cells. Most disease-related membrane receptors or biomarkers are considered to be "overexpressed" on the diseased cells, which implies that they also exist on other cells, only at a lower level. Therefore, overdosage may still damage normal cells and cause side effects. In addition, depending on other factors, such as the stage of the cancer and age of the patients, the optimum dosage of chemotherapy drugs for each patient needs to be determined. A simple and general way to actively adjust the dosage according to the patient's response would be quite beneficial.

One unique feature of aptamers is that their activity can be strongly inhibited by their complementary DNAs (cDNAs) because the complementary base pairing disrupts the aptamer's target-binding conformation. Nucleic acid based antidotes are not only easy to obtain but also have predictable toxicity profiles, which thus eliminates the safety issue associated with antidotes. There have been reports of applying cDNAs as effective inhibitors to deactivate therapeutic aptamers in animals and in humans.[52–54] However, the use of cDNA to inhibit an aptamer's cell-targeting capability as a way to terminate drug delivery has not been demonstrated. Drug carriers such as liposomes can carry many different drug molecules, therefore the cDNA of the targeting aptamer can serve as a "universal" antidote to reduce the efficacy of all of these drugs on the target cells. Neutralized aptamer-based delivery systems lose their disease-targeting capability. Even though cisplatin can still passively diffuse out of the liposomes and into healthy and cancerous cells before the liposome is cleared out of the plasma, the efficiency of nontargeting liposomal drug delivery is usually poor, as demonstrated by the control experiments in the current work, as well as reports from other research labs and clinical trials.[36–38] Therefore, the cDNA-antidote-caused reduction in drug-delivery efficiency could be valuable for reversing adverse drug effects.

We explored whether we could use the cDNA of the aptamer to actively regulate cell uptake of the liposome. Complementary DNA can disrupt the G-quadruplex structure of NCL-aptamer that is critical for the binding to NCL. Therefore, it may be used, when needed, to deactivate the NCL-aptamer on the liposome surface and inhibit cell uptake of the liposomes. Additionally, excess cDNA can be gradually degraded in vivo and rapidly cleared from circulation, so the possibility of cytotoxicity is low. The cDNA employed had a 20-base sequence of 5′-CCA CCA CCA CCA CAA CCA CC-3′, which was composed of all 2′-*O*-methyl-modified RNA bases for improved resistance to nuclease degradation in the cell-culture media. We first tested the concentration dependence of the cDNA on cisplatin delivery and on cell viability. Prior to the addition of the liposomes to MCF-7 cells, different amounts of cDNA (0–10 equiv relative to the NCL-aptamer) were added to the cell culture. The viability of cells treated with NCL-aptamer–liposome–cisplatin was determined by an MTT assay at day 2 and day 4. The study of cDNA-dependent cell viability, as illustrated in Figure 3a, suggested that the cDNA effectively blocked liposome delivery into the cells and led to reduced cell death. The effectiveness of the inhibition of liposome uptake by the cells was proportional to the amount of cDNA added. In the presence of 10 equivalents of inhibitory cDNA, the cell viability remained above 95% after 4 days of incubation of NCL-aptamer–liposome–cisplatin with MCF-7 cells. By contrast, in the absence of cDNA, roughly 50% cell death was observed at day 4. The effectiveness of cDNA for inhibiting aptamer-based drug delivery is demonstrated in vitro; this technique can be useful for fundamental research as well as for practical use, such as the alleviation of overdose or drug-induced allergic reactions.

The effectiveness of antidotes is expected to be related to how quickly they can show their inhibitory effect once they are administered. We thus investigated the time dependence of the cDNA-antidote effect on aptamer-based drug delivery. The antiaptamer cDNA (10 molar equiv relative to the aptamer) was added to the cell culture at different time points during NCL-aptamer–liposome–cisplatin/MCF-7 incu-

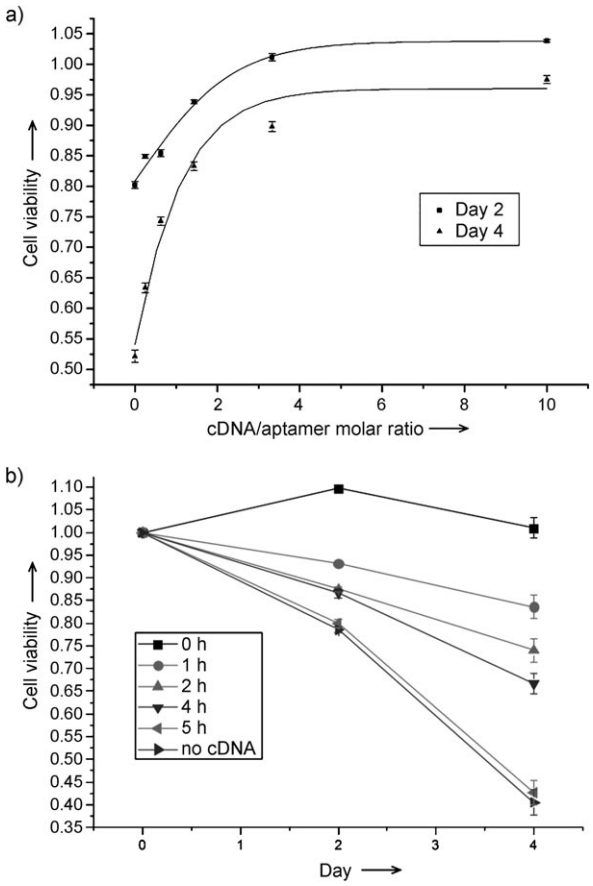

Figure 3. a) Effect of the concentration of the aptamer's cDNA on the viability of MCF-7 cells treated with NCL-aptamer–liposome–cisplatin. b) Time-dependent effect of the cDNA on the viability of MCF-7 cells treated with aptamer–liposome–cisplatin. The cDNA (10 molar equiv relative to the aptamer) was added at different time points during the NCL-aptamer–liposome–cisplatin/MCF-7 incubation.

bation before an MTT assay, and cell viability was evaluated at day 2 and day 4 (Figure 3b). Cells treated with the cDNA at the same time as liposome addition showed the highest viability (approximately 100%) and remained nearly intact throughout the 4 day period, which demonstrated an instantaneous inhibition of the aptamer by the cDNA. When the cDNA was added 1, 2, 4, and 5 h after the addition of NCL-aptamer–liposome–cisplatin to the cells, the cell viabilities decreased to 83.6, 74.0, 66.7, and 42.6%, respectively, most likely caused by cisplatin delivered into the cells before the cDNA was applied.

In conclusion, we have developed aptamer–liposome bioconjugates that can effectively deliver cisplatin in a cancer-cell-specific manner. The poor selectivity of cisplatin was largely overcome, as evidenced by significant killing of the target cancer cells but not the control cancer cells. The formulation of these multifunctional aptamer–liposome–cisplatin conjugates was straightforward and reproducible; the handling of the aptamer-coated liposome drug-delivery system was also much easier than that of an antibody-based system. The binding of the aptamer to the cell-surface receptor can be instantaneously inhibited and disrupted by an antiaptamer cDNA. Our studies provide further support to demonstrate that an aptamer-mediated cancer-targeting strategy is highly specific and can be modulated for desired drug-delivery applications.

Experimental Section

Liposome preparation: Stock solutions of HSPC (1.25 mg), cholesterol, and mPEG2000–DSPE in chloroform were mixed with a 2:1:0.16 molar ratio in a scintillation vial. This mixture was blown dry with N_2 and further dried under vacuum for at least 6 h. The buffer for liposome preparation contained 25 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES; pH 7.6), 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$. This buffer solution (100 μL) containing cholesterol-tagged DNA (3 nmol) was added to the dry lipids. After the mixture had been incubated for 6 h at 37 °C, liposome-preparation buffer (400 μL) containing cisplatin or calcein was added. This solution was gently stirred and kept at 37 °C for 3 h. After that, the solution was stirred and then quickly frozen and thawed for at least 5 cycles. The final solution was incubated at 37 °C overnight. The lipid mixture was then extruded to form liposomes of approximately 200 nm in diameter by following the instructions from Avanti Polar Lipids, Inc. The liposomes were purified on a column packed with Sephadex G-100 medium to remove free cisplatin or calcein.

Cell-specific uptake study by confocal fluorescence microscopy: The MCF-7 and LNCaP cells, cultured as recommended by the American Type Culture Collection (ATCC) in Dulbecco's modified Eagle's medium (DMEM) and Ham's F-12K medium, respectively, were incubated in chamber slides in the medium to allow 70% confluence in 12–24 h. The cell medium was removed. The cells were washed with prewarmed PBS (1×100 μL well^{-1}) and incubated with prewarmed Opti-MEM medium (phenol red reduced) for 30 min before the addition of the liposome solution. Cells were further incubated for 5 h or 10 h at 37 °C. At the prescheduled times, the cells were washed with prewarmed PBS (3×100 μL well^{-1}) and fixed with 4% paraformaldehyde solution for 10 min. The cells were washed again with PBS (1×100 μL well^{-1}) and analyzed with a confocal fluorescence microscopy.

Viability assay: The LNCaP and MCF-7 cells were plated in 96-well plates with the appropriate media at concentrations that allowed 70% confluence in 24 h. On the experiment day, cells were washed with PBS buffer and incubated with Opti-MEM medium for 30 min at 37 °C. After the addition of various formulations of liposomes, the cells were incubated for 5 h and then washed with PBS (2×100 μL well^{-1}). The cells were further incubated in prewarmed growth medium for 48 h or 96 h. The cell viabilities were assessed colorimetrically by following the standard MTT protocol.[55]

Received: March 17, 2009
Revised: June 6, 2009
Published online: July 21, 2009

Keywords: aptamers · cisplatin · drug delivery · liposomes

[1] L. Kelland, *Nat. Rev. Cancer* **2007**, *7*, 573.
[2] E. R. Jamieson, S. J. Lippard, *Chem. Rev.* **1999**, *99*, 2467.
[3] A. Rajeswaran, A. Trojan, B. Burnand, M. Giannelli, *Lung Cancer* **2008**, *59*, 1.
[4] P. J. Bednarski, R. Grunert, M. Zielzki, A. Wellner, F. S. Mackay, P. J. Sadler, *Chem. Biol.* **2006**, *13*, 61.
[5] S. Dhar, Z. Liu, J. Thomale, H. Dai, S. J. Lippard, *J. Am. Chem. Soc.* **2008**, *130*, 11467.

[6] J. W. Park, D. B. Kirpotin, K. Hong, R. Shalaby, Y. Shao, U. B. Nielsen, J. D. Marks, D. Papahadjopoulos, C. C. Benz, *J. Controlled Release* **2001**, *74*, 95.
[7] P. Sapra, T. M. Allen, *Cancer Res.* **2002**, *62*, 7190.
[8] T. A. Elbayoumi, V. P. Torchilin, *Eur. J. Pharm. Sci.* **2007**, *32*, 159.
[9] P. Sapra, T. M. Allen, *Prog. Lipid Res.* **2003**, *42*, 439.
[10] D. B. Kirpotin, D. C. Drummond, Y. Shao, M. R. Shalaby, K. Hong, U. B. Nielsen, J. D. Marks, C. C. Benz, J. W. Park, *Cancer Res.* **2006**, *66*, 6732.
[11] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505.
[12] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818.
[13] M. Blank, T. Weinschenk, M. Priemer, H. Schluesener, *J. Biol. Chem.* **2001**, *276*, 16464.
[14] C. Wang, M. Zhang, G. Yang, D. Zhang, H. Ding, H. Wang, M. Fan, B. Shen, N. Shao, *J. Biotechnol.* **2003**, *102*, 15.
[15] D. A. Daniels, H. Chen, B. J. Hicke, K. M. Swiderek, L. Gold, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15416.
[16] L. Cerchia, F. Duconge, C. Pestourie, J. Boulay, Y. Aissouni, K. Gombert, B. Tavitian, V. de Franciscis, D. Libri, *PLoS Biol.* **2005**, *3*, e123.
[17] B. J. Hicke, C. Marion, Y. F. Chang, T. Gould, C. K. Lynott, D. Parma, P. G. Schmidt, S. Warren, *J. Biol. Chem.* **2001**, *276*, 48644.
[18] S. P. Ohuchi, T. Ohtsu, Y. Nakamura, *Biochimie* **2006**, *88*, 897.
[19] D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang, W. Tan, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11838.
[20] K. T. Guo, R. Schafer, A. Paul, A. Gerber, G. Ziemer, H. P. Wendel, *Stem Cells* **2006**, *24*, 2220.
[21] Z. Tang, D. Shangguan, K. Wang, H. Shi, K. Sefah, P. Mallikratchy, H. W. Chen, Y. Li, W. Tan, *Anal. Chem.* **2007**, *79*, 4900.
[22] D. Shangguan, L. Meng, Z. C. Cao, Z. Xiao, X. Fang, Y. Li, D. Cardona, R. P. Witek, C. Liu, W. Tan, *Anal. Chem.* **2008**, *80*, 721.
[23] H. W. Chen, C. D. Medley, K. Sefah, D. Shangguan, Z. Tang, L. Meng, J. E. Smith, W. Tan, *ChemMedChem* **2008**, *3*, 991.
[24] M. S. Raddatz, A. Dolf, E. Endl, P. Knolle, M. Famulok, G. Mayer, *Angew. Chem.* **2008**, *120*, 5268; *Angew. Chem. Int. Ed.* **2008**, *47*, 5190.
[25] O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie, R. Langer, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 6315.
[26] J. Cheng, B. A. Teply, I. Sherifi, J. Sung, G. Luther, F. X. Gu, E. Levy-Nissenbaum, A. F. Radovic-Moreno, R. Langer, O. C. Farokhzad, *Biomaterials* **2007**, *28*, 869.
[27] V. Bagalkot, L. Zhang, E. Levy-Nissenbaum, S. Jon, P. W. Kantoff, R. Langer, O. C. Farokhzad, *Nano Lett.* **2007**, *7*, 3065.
[28] F. Gu, L. Zhang, B. A. Teply, N. Mann, A. Wang, A. F. Radovic-Moreno, R. Langer, O. C. Farokhzad, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2586.
[29] S. Dhar, F. X. Gu, R. Langer, O. C. Farokhzad, S. J. Lippard, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 17356.
[30] Y. Kaneda, *Adv. Drug Delivery Rev.* **2000**, *43*, 197.
[31] A. Wagner, M. Platzgummer, G. Kreismayr, H. Quendler, G. Stiegler, B. Ferko, G. Vecera, K. Vorauer-Uhl, H. Katinger, *J. Liposome Res.* **2006**, *16*, 311.
[32] M. C. Willis, B. Collins, T. Zhang, L. S. Green, D. P. Sebesta, C. Bell, E. Kellogg, S. C. Gill, A. Magallanez, S. Knauer, R. A. Bendele, P. S. Gill, N. Janjic, *Bioconjugate Chem.* **1998**, *9*, 573.
[33] Y. Matsumura, H. Maeda, *Cancer Res.* **1986**, *46*, 6387.
[34] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, *J. Controlled Release* **2000**, *65*, 271.
[35] J. Woo, G. N. Chiu, G. Karlsson, E. Wasan, L. Ickenstein, K. Edwards, M. B. Bally, *Int. J. Pharm.* **2008**, *349*, 38.
[36] C. Mamot, D. C. Drummond, U. Greiser, K. Hong, D. B. Kirpotin, J. D. Marks, J. W. Park, *Cancer Res.* **2003**, *63*, 3154.
[37] J. M. Meerum Terwogt, G. Groenewegen, D. Pluim, M. Maliepaard, M. M. Tibben, A. Huisman, W. W. ten Bokkel Huinink, M. Schot, H. Welbank, E. E. Voest, J. H. Beijnen, J. M. Schellens, *Cancer Chemother. Pharmacol.* **2002**, *49*, 201.
[38] S. C. White, P. Lorigan, G. P. Margison, J. M. Margison, F. Martin, N. Thatcher, H. Anderson, M. Ranson, *Br. J. Cancer* **2006**, *95*, 822.
[39] C. O. Noble, D. B. Kirpotin, M. E. Hayes, C. Mamot, K. Hong, J. W. Park, C. C. Benz, J. D. Marks, D. C. Drummond, *Expert Opin. Ther. Targets* **2004**, *8*, 335.
[40] P. J. Bates, J. B. Kahlon, S. D. Thomas, J. O. Trent, D. M. Miller, *J. Biol. Chem.* **1999**, *274*, 26369.
[41] M. Srivastava, H. B. Pollard, *FASEB J.* **1999**, *13*, 1911.
[42] M. Derenzini, V. Sirri, D. Trere, R. L. Ochs, *Lab. Invest.* **1995**, *73*, 497.
[43] Y. Otake, S. Soundararajan, T. K. Sengupta, E. A. Kio, J. C. Smith, M. Pineda-Roman, R. K. Stuart, E. K. Spicer, D. J. Fernandes, *Blood* **2007**, *109*, 3069.
[44] K. Xu, R. F. Luduena, *Cell Motil. Cytoskeleton* **2002**, *53*, 39.
[45] S. Soundararajan, W. Chen, E. K. Spicer, N. Courtenay-Luck, D. J. Fernandes, *Cancer Res.* **2008**, *68*, 2358.
[46] V. Dapic, P. J. Bates, J. O. Trent, A. Rodger, S. D. Thomas, D. M. Miller, *Biochemistry* **2002**, *41*, 3676.
[47] D. Kirpotin, J. W. Park, K. Hong, S. Zalipsky, W. L. Li, P. Carter, C. C. Benz, D. Papahadjopoulos, *Biochemistry* **1997**, *36*, 66.
[48] J. W. Park, K. Hong, P. Carter, H. Asgari, L. Y. Guo, G. A. Keller, C. Wirth, R. Shalaby, C. Kotts, W. I. Wood, *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 1327.
[49] J. W. Park, K. Hong, D. B. Kirpotin, G. Colbern, R. Shalaby, J. Baselga, Y. Shao, U. B. Nielsen, J. D. Marks, D. Moore, D. Papahadjopoulos, C. C. Benz, *Clin. Cancer Res.* **2002**, *8*, 1172.
[50] A. Ghezzi, M. Aceto, C. Cassino, E. Gabano, D. Osella, *J. Inorg. Biochem.* **2004**, *98*, 73.
[51] D. R. Budman, A. Calabro, W. Kreis, *Anticancer Drugs* **2002**, *13*, 1011.
[52] C. P. Rusconi, E. Scardino, J. Layzer, G. A. Pitoc, T. L. Ortel, D. Monroe, B. A. Sullenger, *Nature* **2002**, *419*, 90.
[53] C. P. Rusconi, J. D. Roberts, G. A. Pitoc, S. M. Nimjee, R. R. White, G. Quick, Jr., E. Scardino, W. P. Fay, B. A. Sullenger, *Nat. Biotechnol.* **2004**, *22*, 1423.
[54] C. K. Dyke, S. R. Steinhubl, N. S. Kleiman, R. O. Cannon, L. G. Aberle, M. Lin, S. K. Myles, C. Melloni, R. A. Harrington, J. H. Alexander, R. C. Becker, C. P. Rusconi, *Circulation* **2006**, *114*, 2490.
[55] I. Martin-Kleiner, I. Svoboda-Beusan, J. Gabrilovac, *Immunopharmacol. Immunotoxicol.* **2006**, *28*, 411.

