Epidermal growth factor receptor-targeted peptide conjugated phospholipid micelles for doxorubicin delivery

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Abstract

Specific targeting of tumor cells to achieve higher drug levels in tumor tissue and to overcome side effects is the major goal in cancer chemotherapy. In this study, we used a tumor targeting peptide, GE11, to conjugate onto the surface of doxorubicin encapsulated phospholipid micelles. The GE11 peptide triggered specific binding to epidermal growth factor receptor (EGFR), leading to enhanced cellular uptake and cytotoxicity in vitro and highly accumulation in the tumors in vivo. The results indicated that GE11 conjugated phospholipid micelles should have potential applications in cancer therapy.

Keywords

Doxorubicin, epidermal growth factor receptor, GE11, micelles, targeted drug delivery

Introduction

Cytostatic drugs are very useful substances in the fight of various cancers. However, the main disadvantages of conventional chemotherapy are dose limiting side effects because of non-specific action of drugs to other tissues [1]. One possibility to avoid these disadvantages is the development of nano-sized drug carriers [2–4]. Further improvement can potentially be achieved by conjugation of targeting ligands onto nanocarriers to achieve selective delivery to the tumor cell or the tumor vasculature. Various active targeted nanocarriers have been extensively designed and reported [5–7]. Among them, DSPE-PEG2000 [1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)2000] micelles bearing targeting peptides are novel phospholipid nanocarriers for doxorubicin delivery and exhibit good accumulation and penetration in tumor [8–11].

The EGFR is a protein tyrosine kinase encoded by the c-erb-B proto-oncogene and highly expressed on a broad variety of different tumors, including breast, lung, colon, liver, prostate, kidney, head and neck, bladder and ovary carcinoma [12,13]. There exists an association between up-regulation of receptor expression and poor clinical prognosis [14]. Binding of its ligands such as EGF can activate signal transduction pathways that regulate cell proliferation, which will contribute to the transformation of cellular phenotypes and substantial growth of tumor cells [15]. EGFR is a readily accessible cell surface receptor and can serve as a target for selective drug delivery. Many pre-clinical and clinical studies have showed that targeting EGFR is a promising strategy for anticancer therapy [16–19].

The EGFR-antagonist peptide GE11 was identified and isolated in our lab using phage display biopanning [20]. GE11 peptide bound specifically and efficiently to EGFR with a dissociation constant of \(22 \text{nM}\), but with much lower mitogenic activity than with EGF. It has been proved that gene carriers equipped with GE11 bind effectively to EGFR-expressing cancer cells and show transfection efficiencies similar to gene carriers with EGF [21,22]. Targeted gene or drug delivery to EGFR-overexpressing tumors was also achieved by GE11-equipped nanocarriers in vivo [23–26].

To increase cellular uptake and therapeutic efficacy of DSPE-PEG micelles in EGFR-expressing tumors, herein, YC21 (a EGFR targeted oligopeptide composed of GE11 peptide and a GGGSGGGSC linker) was grafted onto the distal end of DSPE–PEG2000–Mal, the extended spacer arm between the maleimide group and GE11 peptide reduces the possibility of steric hindrance [23,27], the resulting DSPE–PEG2000–GE11 was used to construct EGFR-targeted micelles encapsulating doxorubicin. The self-assembly, structures and loading capacity of micelles were assessed. Then in vitro drug release kinetics, cellular uptake and in vitro cytotoxicity were further studied. Finally, in vivo distribution of micelles in a SMMC-7721 xenograft model was evaluated. The aim of our study is to establish the validity.
of using GE11-modified micelles as nanocarriers for cancer therapy.

Materials and methods

Materials

1,2-Distearoyl-sn-glycero-3-phosphoethanolamine–N-[methoxy(polyethylene glycol)2000] (DSPE–PEG2000), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine–N-[maleimide (polyethylene glycol)2000] (DSPE–PEG2000–Mal) were purchased from NFC Corporation (Japan). The YC21 (GE11) peptide (Sequence: NH₂-Tyr-His-Trp-Tyr-Gly-Tyr-Thr-Pro-Gln-Val-Lie-Gly-Gly-Ser-Gly-Gly-Ser-Cys-COOH, molecular weight 2160) was synthesized by GL Biochem (Shanghai, China). Doxorubicin hydrochloride was supplied by Higherbio Co., Ltd. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Shanghai Jingchun reagent Co., Ltd. (Shanghai, China). 1,1'-diodoacetetyl-3,3',3'-tetramethyl indotricarbocyanine iodide (DiR) was obtained from Biotium, Inc. (Hayward, CA). Triethylamine, Dimethylsulfoxide, 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and other chemicals in analytical grade were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Human hepatoma cell line SMMC-7721 was cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) culture medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (100 mg/ml streptomycin and 100 U/ml penicillin), MCF-7 cells (ATCC) were maintained in DMEM with 20% FBS and 1% antibiotic solution. All cells were cultured in a humidified atmosphere containing 5% CO₂ at 37°C.

Athymic female mice (BALB/c strain; 4–6 weeks, 15–17 g weight) were purchased from the Shanghai slac laboratory animal Co., Ltd. (Shanghai, China) and maintained in a SPF environment under controlled temperature (24°C). Animal experiments were performed in accordance with the CAPN (China Animal Protection Law) and protocols were approved by the Shanghai JiaoTong University Animal Care and Use Committee.

Synthesis of DSPE–PEG2000–GE11

DSPE–PEG2000–Mal was dissolved in 50 mM HEPES buffer (pH 6.5), then peptide YC21 powder was added (molar ratio of DSPE–PEG2000–Mal: YC21 = 1:1.1), the mixture was stirred for 48 h under room temperature, the reaction solution was dialyzed against distilled water with a molecular weight cut-off of 3500 Da and lyophilized.

Preparation of micelles

Doxorubicin-loaded micelles (DM) were prepared from DSPE–PEG2000 and doxorubicin via the film dispersion method. Briefly, 1 mg/ml doxorubicin hydrochloride in methanol solution was alkaliyzed by triethylamine for 15 min at room temperature (molar ratio of doxorubicin: triethylamine = 1:2), then mixed with 10 mg/ml DSPE–PEG2000 in chloroform at a molar ratio of 1:1. The solvent was removed by vacuum rotary evaporation to form a dry drug-containing lipid film which was subsequently hydrated with HEPES buffer (20 mM; pH 7.2) at 60°C for 2 h. Non-encapsulated doxorubicin was separated by filtration of the micelle suspension through a 220nm nylon membrane (Rephile bioscience Ltd, Boston, MA). GE11-modified doxorubicin-loaded micelles (GE11-DM) were prepared in an identical procedure except that DSPE–PEG2000 was replaced with a mixture of DSPE–PEG2000–GE11 and DSPE–PEG2000 (molar ratio 5:1). For in vivo fluorescent imaging, DiR was loaded into micelles at a concentration of 5 μg/ml. The DiR-loaded micelles were prepared using the same procedures as DM.

Characterization of micelles

The average size and zeta potential of micelles were measured by dynamic light scattering (DLS; ZetaSizer Nano ZS90, Malvern Instrument, Westborough, MA). The diameter mean values were calculated from the measurements performed in triplicate. Doxorubicin loaded inside the micelles was determined by UV absorption and the drug-loading content (DLC wt%) and the drug-loading efficiency (DLE, wt%) were calculated by the following equations:

\[ \text{DLC} = \frac{\text{Amount of doxorubicin in micelles}}{\text{Amount of doxorubicin loaded in micelles}} \times 100\% \]

\[ \text{DLE} = \frac{\text{Amount of doxorubicin in micelles}}{\text{Amount of doxorubicin for micelles preparation}} \times 100\% \]

The morphology of micelles was observed using transmission electron microscopy (TEM; Tecnai G2 Spirit Biotwin, FEI, Hillsboro, OR) without negative staining, one drop of micelle solution (1 mg/ml) was placed on a copper grid and air-dried before measurements.

In vitro drug release

The in vitro release of doxorubicin from DM and GE11-DM was determined by a dialysis method. About 100 μl of DM or GE11-DM (doxorubicin concentration: 0.9 mg/ml) was dialyzed against PBS buffer (pH 7.4 and pH 5.0) using a dialysis membrane with molecular weight cut-off of 7000 Da at 37°C and shaken at 100 rpm. At 1, 2, 3, 5, 7, 9, 12, 24, 36 and 48 h, 700 μl aliquot of the medium was withdrawn and 700 μl of fresh release medium was added. The concentration of doxorubicin in the samples was determined by measuring optical density at 498 nm using the powerwave XS Spectrophotometer (Bio-Tek, Winooski, VT) and cumulative release was calculated and plotted against time.

Particle cellular uptake studies and competition assay

Cellular uptake and distribution of doxorubicin from DM and GE11-DM were observed by confocal laser scanning microscopy (CLSM). SMMC-7721 or MCF-7 cells were seeded onto culture slides in 24-well plates at a density of 30,000 cells per well and allowed to grow for 18 h at 37°C. Cells were incubated for 30 min with DM or GE11-DM (equivalent doxorubicin concentration: 10 μg/ml). After removing the micelles and washing the wells twice with PBS, the cells were
fixed by 4% paraformaldehyde for 30 min and the cell nuclei were then stained with DAPI for 10 min. The cells were mounted and observed under confocal laser scanning microscopy (Leica Confocal TCS SP8 MP, Wetzlar, Germany), the following wavelengths were used: excitation at 480 nm and detection through a 560 nm filter for doxorubicin, and excitation at 350 nm and detection through a 460 nm filter for DAPI.

Flow cytometry was also used to examine the cellular uptake of DM and GE11-DM. In total, 200 000 cells per well were seeded into 12-well plates the night before treatment, then cultured with DM or GE11-DM, or free doxorubicin (equivalent doxorubicin concentration of 10 μg/ml) for 30 min, the cells were washed twice with PBS, trypsinized and centrifuged at 1000 rpm for 5 min, precipitated cells were then suspended in a flow cytometry buffer (1% FBS in PBS) and analyzed using a flow cytofluorometer (BD FACSCalibur, San Jose, CA).

To confirm the specificity internalization of GE11-DM for EGFR, a competition assay was conducted on SMMC-7721 cells as described previously [11]. Briefly, SMMC-7721 cells were plated onto culture slides in 24-well plates and grown overnight in complete medium. Cells were incubated in serum-free medium for 30 min, blocked with 5% BSA (diluted with serum-free medium) for 30 min, then pre-incubated with 0.1 mg/ml of anti-EGFR MAb C225 (cetuximab) for 15 min in serum-free medium. The cells were washed with PBS and incubated with GE11-DM at a final doxorubicin concentration of 10 μg/ml for 30 min, cellular uptake was analyzed by confocal microscopy and flow cytometry.

Cytotoxicity assay

In vitro cytotoxicity of DM and GE11-DM was evaluated by MTT assay with SMMC-7721 cells and MCF-7 cells. Cells were seeded in 96-well plates at a density of 5000 cells per well in 100 μl of complete culture medium and incubated for 18 h attachment, the medium was then replaced by DM or GE11-DM in medium at various doxorubicin concentrations ranging from 0.1 to 25 μg/ml. After 24 h, the medium was replaced with 100 μl of 0.5 mg/ml MTT in culture medium and 3 h later the MTT solution was replaced with 150 μl of DMSO solution. The plates were shaken at 100 rpm for 15 min at 37 °C before the relative color intensity was measured at the analysis wavelength of 490 nm using a powerwave XS microplate reader (Bio-Tek, Winooski, VT). Cells without treatment were used as the control and all experiments were carried out with four replicates (n = 4).

Apoptosis and cell cycle analysis

In total, 150 000 SMMC-7721 cells per well were seeded in 6-well plates and incubated for 18 h attachment. After 24 h of incubation with DM or GE11-DM in medium (final concentration of doxorubicin: 1 μg/ml) at 37 °C, cell apoptosis assay was performed using FITC Annexin V Apoptosis Detection Kit (Beyotime, Jiangsu, China) according to the manufacturer’s protocol. The fluorescence-stained SMMC-7721 cells were analyzed by flow cytometer (BD FACSCalibur, San Jose, CA).

To analyze the cell cycle distribution, 400 000 SMMC-7721 per well were seeded in 6-well plates and incubated for 18 h attachment, then the medium was replaced with DM or GE11-DM in medium (final concentration of doxorubicin: 1.5 μg/ml) and cells were incubated for an additional 24 h. Cell cycle was detected using Propidium Iodide Kit (Beyotime, Jiangsu, China) according to the manufacturer’s protocol and analyzed by flow cytometry.

In vivo imaging and biodistribution

SMMC-7721 cells (3 × 10^6 cells) suspended in 200 μl of serum-free medium were subcutaneously inoculated on the right fore-limbs of female BALB/c nude mice. About 14 days post-inoculation and when the tumors reached a volume of about 200–350 mm^3, mice were randomly assigned to

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**Figure 1.** Schematic illustration of the formation and internalization of self-assembled doxorubicin-loaded GE11-modified micelles.

**DSPE-PEG2000**

**DSPE-PEG2000-GE11**

**Doxorubicin**

**enhanced internalization**

**cancer cell**

**EGFR**
treatment groups: 3 mice per group × 3 groups, phosphate buffer saline, 10 μg/kg of DiR-loaded micelles and targeted micelles were injected through the caudal vein. After 2, 4, 8 and 24 h respectively, the mice were anaesthetized and imaged using an IVIS Lumina II in vivo imaging system (Caliper Life Sciences, Hopkinton, MA) with an excitation bandpass filter at 730 nm and an emission at 790 nm. After living imaging, mice were sacrificed and tumors and other tissues (liver, kidney, spleen, heart and lung) were isolated and immediately scanned again with the same system as described above.

**Statistical analysis**

Student’s two-tailed t-test was used to calculate p values. p < 0.05 was considered significant.

**Results and discussion**

**Preparation and characterization of micelles**

The preparation strategy for DM and GE11-DM is shown in Figure 1. First, DSPE–PEG2000–GE11 was synthesized by coupling the thiol group of YC21 to the maleimide group of DSPE–PEG2000–Mal, then the DM and GE11-DM were prepared by a film dispersion method. The particle size and zeta potential were determined by dynamic light scattering. The diameter of DM was 11.4 ± 0.3 nm, and the zeta potential was 0.3 ± 0.2 mV, while the diameter of GE11-DM was 13.0 ± 1.0 nm and the zeta potential was −5.1 ± 1.0 mV. The particle size results measured by DLS were consistent with TEM images as shown in Supplementary Figure S1, both DM and GE11-DM aggregated to the uniformly spherical morphology in a neutral environment with a narrow size distribution.

**Drug loading level and in vitro drug release**

The amount of doxorubicin incorporated into the micelles was measured by UV analysis using the absorption peak of doxorubicin at 498 nm after adding 180 μl of acidic isopropanol (81 mmol/L HCl) to 20 μl of drug loaded micelles [28], the drug-loading content (DLC) value of DM was 17 wt% with a drug-loading efficiency (DLE) of 98%, while DLC...
value of GE11-DM was 15 wt% and DLE was 99%. To study the effects of pH values on the drug release rates of the doxorubicin-loaded micelles, *in vitro* drug release studies were performed by a dialysis method under simulated physiological (pH 7.4) and acidic intracellular (pH 5.0) conditions at 37 °C. As shown in Figure 2, the pH value of the medium had a considerable effect on the release rate of doxorubicin from the micelles. The release rate of doxorubicin was much higher at a pH of 5.0 than at a pH of 7.4 and the release profiles of DM and GE11-DM were similar at each pH. The faster release of doxorubicin in acidic conditions is likely due to the reprotonation of the amino group of doxorubicin. Given that micellar particles are usually internalized inside the cells by endocytosis [29], an accelerated release inside the endosome/lysosome of tumor cells may occur due to the decreased pH value.

**Cell uptake studies**

Cellular internalization of DM and GE11-DM were studied using both confocal microscopy and flow cytometry. SMMC-7721 cells were used as EGFR-positive cells while MCF-7 cells as EGFR-negative cells [30]. The mean fluorescence intensity (MFI) was given to make a quantitative comparison of the endocytosis of doxorubicin because the fluorescence intensity is proportional to the amount of doxorubicin internalized by the cells.

To assess the optimal molar rations of lipids, we prepared GE11-DM in various molar rations (DSPE–PEG2000–GE11:DSPE–PEG2000 = 1:1, 3:1, 5:1, 7:1 and 9:1) and investigated cellular uptake in SMMC-7721 cells. Our results showed maximum cellular uptake with the molar ration of 5:1, which was used as a model in most cases of the studies (Supplementary Figure S2).

Flow cytometry histograms are shown in Figure 3. In SMMC-7721 cells, the level of cellular uptake decreased in the following order: GE11-DM, DM and free doxorubicin, the MFI value of GE11-DM was 1.4 times higher than that of DM and 2.3 times higher than that of free doxorubicin. While in MCF-7 cells, there was no significant difference in fluorescence intensity between GE11-DM and DM. These results indicated that a receptor-mediated endocytosis was involved in the cellular uptake of GE11-DM. To further verify the cellular uptake behavior of DM and GE11-DM, the confocal microscopy analysis was performed. As shown in Figure 4, similar to the findings observed using flow cytometry, the

![Figure 4](image-url)
fluorescence intensity of doxorubicin from GE11-DM was stronger than that from DM in SMMC-7721 cells. Doxorubicin delivered by the DM and GE11-DM were largely localized in the cytoplasm and cell nuclei which may be due to the pH sensitivity property of micelles, after micelles entered into endosomes (pH 5.0–6.0) or lysosomes (pH 4.0–5.0), doxorubicin could easily escape from micelles and enter the cytoplasm and cell nuclei [31,32].

To confirm the specific endocytosis of GE11-DM through EGFR, a competition assay was conducted. As shown in Figure 3(A) and Figure 4, in the case of SMMC-7721 cells were pre-treated with excess anti-EGFR monoclonal antibody C225 (cetuximab) for 15 min, the fluorescence intensity of doxorubicin from GE11-DM was dramatically reduced and became largely equivalent to that of the DM. Both the confocal microscopy and flow cytometry results indicated that GE11-DM was taken up through EGFR-mediated endocytosis.

Cytotoxicity study
SMMC-7721 and MCF-7 cells were selected for cytotoxicity tests of DM and GE11-DM. SMMC-7721 and MCF-7 cells were used as EGFR-positive and EGFR-negative cell lines respectively. Cell viability (%) was measured after incubation with DM and GE11-DM for 24 h in the two cell lines (Figure 5), a lower IC50 value for SMMC-7 cells was observed for GE11-DM (0.6 μg/ml) when compared to that of DM (1.8 μg/ml). However, there was little difference in the IC50 when MCF-7 cells were treated with DM (1.2 μg/ml) and GE11-DM (1.5 μg/ml). The enhanced cytotoxicity of

![Figure 5](image-url) **Figure 5.** For in vitro cytotoxicity studies, SMMC-7721 (A) and MCF-7 (B) cells were treated with DM and GE11-DM at varying concentrations. Cell viability was analyzed by MTT assay and calculated as a percentage of living cells. Each point represents the mean of four experiments. Data are shown as the mean ± SD (n = 4).

![Figure 6](image-url) **Figure 6.** Antiproliferative activity of DM and GE11-DM against SMMC-7721 cells after 24 h incubation, cell apoptotic and necrotic rates were detected by flow cytometry. The horizontal and vertical axes represent labeling with Annexin V-FITC and propidium iodide (PI), respectively.
GE11-DM in SMMC-7721 cells might be attributed to their selective binding to the EGFR expressed on the surface of cells. These cytotoxicity results suggest that anti-EGFR peptide GE11-modified doxorubicin-loaded micelles could increase the selectivity for killing cancer cells with EGFR overexpression.

**Apoptosis and cell cycle analysis**

To explore whether the viability decline of cells treated by various doxorubicin formulations is related to the apoptosis induced by doxorubicin, Annexin V-PI staining assay was performed on SMMC-7721 cells. As shown in Figure 6, in the group treated with GE11-DM, the percentage of early apoptotic, late apoptotic and necrotic cells increased when compared with that of the group incubated with DM. The increased apoptotic activity of GE11-DM is probably due to the higher level of cell uptake through receptor-mediated endocytosis.

The cell cycles of SMMC-7721 cells treated with DM and GE11-DM were analyzed as shown in Figure 7, compared to untreated control cells, a marked increase of G2/M phase arrest was observed in both DM and GE11-DM treated cells. For the treatments of culture medium, DM and GE11-DM, 9.2, 12.4 and 13.5% cells were arrested in G2/M phase after 24 h, respectively. There was no obvious difference between DM and GE11-DM in cell cycle arrest performance.

![Figure 7. Variation in cell cycle of SMMC-7721 cells after treatment with DM and GE11-DM for 24 h.](Figure 7. Variation in cell cycle of SMMC-7721 cells after treatment with DM and GE11-DM for 24 h.)
In vivo tumor-targeting observed by NIRF imaging

The biodistribution and tumor targeting efficiency of various micellar formulations were evaluated using a non-invasive near infrared optical imaging technique. Figure 8(A) shows the real-time whole-body fluorescence at 2, 4, 8 and 24 h post-injection of phosphate buffer saline (control), DiR-loaded micelles and GE11-modified DiR-loaded micelles in the SMMC-7721 armpit tumor bearing nude mouse model. For both micellar formulations, a strong DiR fluorescence was detected in most organs of the mice, resulting from long circulation of micelles in the bloodstream. Visible fluorescence accumulation appeared in the tumors of both micellar formulations group since 2 h post-injection, the fluorescence signal intensity of the GE11-modified micelles group in the tumor region was stronger than that of unmodified micelles group and this pattern maintained during the entire monitoring period. In quantitative analyses (Figure 8B), GE11-modified micelles exhibited higher intensity at the tumor tissue than that of unmodified micelles at all time-points examined.

The biodistribution of micelles in tumor xenograft mice was also evaluated by ex vivo imaging. Mice were sacrificed at 24 h post-injection and ex vivo fluorescent images of tumors, organs and blood were obtained. As shown in Figure 8(C), a higher fluorescence accumulation in the tumors of GE11-modified micelles group was detected when compared to that of passive unmodified micelles group. Strong fluorescence was also observed in liver tissues of both groups, which should be ascribed to the reticuloendothelial system.

The following two key factors may contribute to the high tumor targeting ability of GE11-modified DiR-loaded micelles: first, it has been reported that long circulation in the blood could be one of the most significant factors determining tumor targeting efficiency [33]. GE11-modified DiR-loaded micelles with hydrophilic surface formed by polyethylene glycol can escape macrophage capture effectively [34]. Second, the binding ability of GE11 to EGFR facilitates faster cellular uptake and leads to the active tumor targeting of micelles. Overall, the biodistribution studies are in line with our previous studies on the biodistribution of GE11-modified micelles [28].

Figure 8. Representative time-dependent whole body images of SMMC-7721 bearing female BALB/c nude mice after intravenous injection of PBS or DiR-loaded micelles, similar results were observed in the other two repeat experiments. (B) Average fluorescence intensity of tumors 2, 4, 8 and 24 h after DiR treatment. The asterisk (*) represents data points that have significant difference (p < 0.05; two-tailed Student’s t tests). (C) Ex vivo optical imaging of tumors, organs and blood of SMMC-7721 bearing female BALB/c nude mice sacrificed at 24 h after DiR treatment.
indicate that GE11-modified micelles could be a highly efficient doxorubicin delivery vehicle for active targeting of EGFR-positive cancer cells in vivo.

Conclusions

In summary, the EGFR-antagonist peptide GE11-modified doxorubicin-loaded DSPE–PEG micelles exhibited enhanced cellular uptake and cytotoxic effects in EGFR-expressing cells due to receptor-mediated endocytosis. In tumor-bearing nude mice, GE11-modified DiR-loaded micelles also exhibited a much higher level of DiR uptake in tumor tissue than nontargeted micelles according to NIRF imaging. Both the in vitro and in vivo data proved the GE11-modified DSPE–PEG micelles could be a promising EGFR-targeted delivery system to enhance the intracellular delivery of doxorubicin.

Declaration of interest

The authors report no declarations of interest. This work was supported by the Supporting Program of the “Twelfth Five-year Plan” for Science and Technology Research of China (Grant No. 2012ZX10002014-006), National Natural Science Foundation of China (No. 81302708), Medical-Engineering Joint Funds of Shanghai Jiao Tong University (No. YG2013MS41) and Innovation Fund from Shanghai Cancer Institute (No. SB14-05).

References

1. Schlitt A, Jordan K, Vordermark D, et al. Cardiotoxicity and oncological treatments. Dtsch Arztebl Int 2014;111:161–8.
2. Ruoslahi E, Bhata SN, Sailor MJ. Targeting of drugs and nanoparticles to tumors. J Cell Bioi 2010;188:759–68.
3. Steichen SD, Caldonera-Moore M, Peppas NA. A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics. Eur J Pharm Sci 2013;48:416–27.
4. Mohri K, Nishikawa M, Takahashi Y, Takakura Y. DNA nanotechnology-based development of delivery systems for bioactive compounds. Eur J Pharm Sci 2014;58:26–33.
5. Song S, Liu D, Peng J, et al. Peptide ligand-mediated liposome distribution and targeting to EGFR expressing tumor in vivo. Int J Pharm 2008;363:155–61.
6. Tan ML, Choong PF, Dass CR. Recent developments in liposomes, microparticles and nanoparticles for protein and peptide drug delivery. Peptides 2010;31:184–93.
7. Zhang G, Zeng X, Li P. Nanomaterials in cancer-therapy drug delivery system. J Biomed Nanotechnol 2013;9:741–50.
8. Tang N, Du G, Wang N, et al. Improving penetration in tumors with nanoassemblies of phospholipids and doxorubicin. J Natl Cancer Inst 2007;99:1004–15.
9. Kastantin M, Ananthanarayanan B, Karmali P, et al. Effect of the lipid chain melting transition on the stability of DSPE-PEG(2000) micelles. Langmuir 2009;25:7279–86.
10. Wang Y, Wang R, Lu X, et al. Pegylated phospholipid-based self-assembly with water-soluble drugs. Pharm Res Dordr 2010;27:361–70.
11. Wei T, Liu J, Ma H, et al. Functionalized nanoscale micelles improve drug delivery for cancer therapy in vitro and in vivo. Nano Lett 2013;13:2528–34.
12. Xu YH, Richert N, Ito S, et al. Characterization of epidermal growth factor receptor gene expression in malignant and normal human cell lines. Proc Natl Acad Sci USA 1984;81:7308–12.
13. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Bio 2001;2:127–37.
14. Nicholson R, Gee J, Harper M. EGFR and cancer prognosis. Eur J Cancer 2001;37:9–15.
15. Khutz K, Schaffert D, Willhauck MJ, et al. Epidermal growth factor receptor-targeted (131)I-therapy of liver cancer following systemic delivery of the sodium iodide symporter gene. Mol Ther 2011;19:676–85.
16. Mendelsohn J, Baselga J. Status of epidermal growth factor receptor antagonists in the biology and treatment of cancer. J Clin Oncol 2003;21:2787–99.
17. Julien DC, Behnke S, Wang G, et al. Utilization of monoclonal antibody-targeted nanomaterials in the treatment of cancer. mAbs 2011;3:467–78.
18. Medina OP, Pillarsetty N, Glekas A, et al. Optimizing tumor targeting of the lipopholic EGFR-binding radiotracer SKI 243 using a liposomal nanoparticle delivery system. J Control Release 2011;149:292–8.
19. Yuan Y, Chen S, Pauensk T, et al. Epidermal growth factor receptor targeted nuclear delivery and high-resolution whole cell X-ray imaging of FeO₃/TiO₂ nanoparticles in cancer cells. ACS Nano 2013;7:10502–17.
20. Li Z, Zhao R, Wu X, et al. Identification and characterization of a novel peptide ligand of epidermal growth factor receptor for targeted delivery of therapeutics. FASEB J 2005;19:1978–85.
21. Schafer A, Pahnke A, Schaffert D, et al. Disconnecting the yin and yang relation of epidermal growth factor receptor (EGFR)-mediated delivery: a fully synthetic, EGFR-targeted gene transfer system avoiding receptor activation. Hum Gene Ther 2011;22:1463–73.
22. Abourbeh G, Shir A, Mishani E, et al. PolyIC GE11 polyplex inhibits EGFR-overexpressing tumors. IUBMB Life 2012;64:324–30.
23. Liu M, Li ZH, Xu EJ, et al. An oligopeptide ligand-mediated therapeutic gene nanocomplex for liver cancer-targeted therapy. Biomaterials 2012;33:2240–50.
24. Ohno S, Takanashi M, Sudo K, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther 2013;21:185–91.
25. Kopansky E, Shamay Y, David A. Peptide-directed HPMA copolymer-doxorubicin conjugates as targeted therapeutics for colorectal cancer. J Drug Target 2011;19:533–43.
26. Liu CW, Lin WJ. Using doxorubicin and siRNA-loaded heptapeptide-conjugated nanoparticles to enhance chemosensitization in epidermal growth factor receptor high-expressed breast cancer cells. J Drug Target 2013;21:776–86.
27. Stefanick JF, Ashley JD, Bilgicer B. Enhanced cellular uptake of peptide-targeted nanoparticles through increased peptide hydrophilicity and optimized ethylene glycol peptide-linker length. ACS Nano 2013;7:8115–27.
28. Lee TY, Wu HC, Tseng YL, Lin CT. A novel peptide specifically binding to nasopharyngeal carcinoma for targeted drug delivery. Cancer Res 2004;64:8002–8.
29. Nam HY, Kwon SM, Chung H, et al. Cellular uptake mechanism and intracellular fate of hydrophilically modified glycol chitosan nanoparticles. J Control Release 2009;135:259–67.
30. Jiang H, Wang H, Tan Z, et al. Growth suppression of human hepatocellular carcinoma xenografts by a monoclonal antibody CH12 directed to epidermal growth factor receptor variant III. J Biol Chem 2011;286:5913–20.
31. Ganta S, Devalapally H, Shahiwala A, Amiji M. A review of stimuli-responsive nanocarriers for drug and gene delivery. J Control Release 2008;126:187–204.
32. Shenoy D, Little S, Langer R, Amiji M. Poly(ethylene oxide)-modified poly(beta-amino ester) nanoparticles as a pH-sensitive system for tumor-targeted delivery of hydrophobic drugs. 1. In vitro evaluations. Mol Pharmaceut 2005;2:357–66.
33. Papahadjopoulos D, Allen TM, Gabizon A, et al. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeuetic efficacy. Proc Natl Acad Sci USA 1991;88:11460–4.
34. Torchilin VP. PEG-based micelles as carriers of contrast agents for different imaging modalities. Adv Drug Deliver Rev 2002;54:235–52.