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To link to this article: http://dx.doi.org/10.3109/1061186X.2015.1070857

Published online: 31 Jul 2015.

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Magnetic albumin immuno-nanospheres as an efficient gene delivery system for a potential use in lung cancer: preparation, in vitro targeting and biological effect analysis

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Abstract
Magnetic albumin immuno-nanospheres (MAINs), simultaneously loaded with super-paramagnetic iron oxide nanoparticles for targeting application and anticancer gene, plasmid-survivin/shRNA (pshRNA) and modified with anti-EGFR monoclonal antibody Cetuximab for targeting and treatment agents, were prepared for targeting lung cancer. Transmission electron microscopy images and transfection photographs, respectively, showed that magnetic nanoparticles and pshRNA were successfully encased in the albumin nanospheres. The release profiles in vitro indicated that nanospheres had an obvious effect of sustained release of pshRNA. The results of slide agglutination test and immunofluorescence analysis demonstrated that the immuno-nanospheres retained the immuno-reactivity of Cetuximab. The MAINs significantly increased adherence and uptake by GLC-82 lung cancer cells over-expressed epidermal growth factor receptor over a magnetic albumin nanospheres (MANs) control. The pshRNA-loaded MAINs formulation was more effective than equimolar doses of free Cetuximab, single magnetic targeting with pshRNA (pshRNA-loaded MANs) or single monoclonal antibody targeting with pshRNA (pshRNA-loaded AINs) in the treatment of GLC-82 lung cancer cells. Collectively, the study indicates that the novel pshRNA-loaded magnetic immuno-nanospheres represent a promising approach for magnetic and monoclonal antibody-dependent gene targeting in lung cancer therapy.

Keywords
Cetuximab, magnetic albumin immuno-nanospheres, survivin/shRNA

History
Received 13 April 2015
Accepted 6 July 2015
Published online 31 July 2015

Introduction
RNA interference (RNAi) technology to specifically eliminate or shut down gene expression is coming forth as a potential strategy to treat cancer. Because of the unstable and rapidly degrading property of single siRNA [1], the shRNA silencing specific gene expression is usually connected to the vector. The success of gene therapy relies on the effectiveness of available vehicles for gene delivery. Cancer therapy requires the selective delivery of antineoplastic agents to a local tumor and more particularly to metastases in the body. Targeting-nonviral vectors are considered advantageous for gene therapy due to their ability to minimize the host immune response compared with viral vectors, improve the biological index of highly active agents and accuracy of gene delivery to target-tissues.

In recent years, magnetic albumin nanospheres (MANs) comprising maghemite nanoparticle hosted in polymeric albumin nanospheres have caused broad concern in the delivery system research field. The endogenous nature of albumin without hemolytic and immunogenic problems might render albumin nanospheres delivery system highly efficient and safe as a carrier for drugs [2–4]. Excitingly, albumin paclitaxel nanoparticle (Abraxane) was approved by the FDA in 2005 for the treatment of metastatic breast cancer through preclinical as well as clinical extensive investigations, recently it was also approved for the treatment of non-small-cell lung cancer. These factors may be fine-tuned for nucleic acid delivery system, such as DNA [5] and siRNA [1]. Super-paramagnetic iron oxide nanoparticles (SPIONs) have been applied in biomedical research and clinical applications as an effective drug and gene delivery system, attribute to their specific magnetic properties, biocompatibility, stability in body fluids and non-immunogenicity [6]. The SPIONs aggregate only in the presence of a strong external magnetic field, however, after the period field is removed, the magnetization disappears [7]. They have the advantage of being used in biomedical applications such as drug [8] and gene delivery [9,10], magnetic-targeting, hyperthermia [11], magnetic resonance imaging and cancer diagnosis.
Hence, SPIONs encapsulated in albumin nanospheres (MANs) might render the nanosphere delivery system suitable for targeting under an applied magnetic field. MANs have been successfully used to load antitumor drugs, for example doxorubicin [12] and Gemcitabine [13] and the loaded-drug nanospheres showed enhanced antitumor efficacy compared with free drug.

With the rapid development of monoclonal anticancer antibodies in the pharmaceutical industry, an increasing number of research groups have conjugated the monoclonal antibody inhibiting cancer proliferation, migration or angiogenesis to the surface of nanocarriers, including inorganic TiO$_2$ [14], gold [15] and magnetic particles [16], also biopolymers PEGy-lated liposomes [17], poly(D,L-lactide-co-glycolide) nanoparticles [18] and albumin nanospheres [19]. Among these, magnetic albumin immuno-nanospheres (MAINs), composed of magnetic materials encapsulated into the albumin nanospheres and modified by monoclonal antibody, are defined as a potential candidate for high efficient drug or gene delivery. This is not only due to the targeting achieved using external magnetic guidance, but also due to the capacity of antibodies to attach to their surfaces. Epidermal growth factor receptor (EGFR) is a cell surface receptor that plays an important role in signaling pathways and is able to regulate cell proliferation, angiogenesis and tumor metastases. This receptor is widely over-expressed in certain types of tumors such as lung cancer [20] and metastatic colorectal cancer [21], which makes it a key biological target for anticancer therapy. Cetuximab (marketed as Erbitux), a chimeric human/mouse immunoglobulin G1 antibody that binds specifically to the extracellular domain of human EGFR, was approved by the US Food and Drug Administration in 2004 as a second-line treatment for advanced colorectal cancer. However, there are few reports on the application of Cetuximab conjugated to nanospheres.

Survivin, a member of the inhibitor of apoptosis protein family [22], is over-expressed in many human carcinomas while undetected in most normal adult tissues [23]. In this study, we constructed shRNA recombinant expression vector targeting survivin gene, then prepared a novel tumor-targeting nanospheres (pshRNA-loaded MAINs) by desolvation-crosslinking method. The nanospheres consist of SPIONs Fe$_3$O$_4$ and plasmid carrying shRNA to survivin hosted in bovine albumin nanospheres, in addition to cetuximab conjugated to the albumin nanospheres through the heterobifunctional crosslinkers SPDP. To characterize the novel nanospheres, several parameters were studied, including gene release and transfection in vitro. The targeting capability to the lung cancer cell was explored by immunofluorescence assay, Prussian blue staining and direct scanning electron microscope observation. In addition, the MTT assay and flow cytometry analysis were performed to observe the antitumor efficacy of these targeting nanospheres to lung cancer cells.

**Main reagents and cell lines**

Bovine serum albumin (BSA), SPDP (N-succinimidyl-3-(2-pyridyldithiol)propionate) and DTT (DL-dithiothreitol) were purchased from Sigma-Aldrich (St. Louis, MO), plasmid pGPU6/GFP/Neo-shRNA (pshRNA) were synthesized by GenePharma (Shanghai, China). Human lung cancer GLC-82 cell lines were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institute of Biological Sciences, Chinese Academy of Sciences (Shanghai, China).

**Methods**

**Construction and amplification of the survivin-shRNA plasmid expression vector**

Double chains of oligonucleotide with complementary sequences, survivin-shRNA plasmid that can code short hairpin RNA (shRNA), were obtained from GenePharma Co., Ltd. The vector of survivin-shRNA plasmid is pGPU6/GFP/Neo including green fluorescent protein gene, which can serve as one report gene in the experiments. In order to get more plasmids for the experiments, the plasmid was transformed into Escherichia coli DH5α cells, and then planted on solid LB medium. Kanamycin-resistant colonies were cultured in LA medium, which were shaken overnight in a 37°C rocking bed; then the recombinant survivin-shRNA plasmid was prepared and stored at −20°C.

**Preparation and characterization of Fe$_3$O$_4$ magnetic nanoparticles**

Fe$_3$O$_4$ magnetic nanoparticles were prepared by the technique of chemical co-precipitation [for details, see 24] and its shape and diameter were observed by a transmission electron microscope (TEM) (JEM-200CX, Tokyo, Japan).

**Preparation of the albumin nanospheres**

pshRNA-survivin-loaded albumin nanospheres (pshRNA-ANS) and their magnetic albumin nanospheres (pshRNA-MANS) were prepared by a desolvation-crosslinking method. Briefly, 3 mL of an aqueous mixture of 100 mg BSA and 50 mg plasmid-shRNA, with or without 40 mg Fe$_3$O$_4$ (prepared previously) nanoparticles was sonicated in distilled water. Then, added the 1 M NaOH solution until the PH of the aqueous mixture was adjusted to 9.0, appropriate absolute alcohol was dripped by the speed of 1 mL/min until the solution appeared gelatinous. The resultant emulsion was treated with 50 μL 0.25% glutaraldehyde as a crosslinking agent, the crosslinking process was performed with overnight stirring of the suspension. The nanospheres solution were centrifuged at 15 000 rpm, supernatant was outwelled, the sediments were washed three times by distilled water. The control albumin nanospheres (ANS) were also prepared by using the same procedure with aqueous BSA solution but without pshRNA.

**Preparation of MAINS**

The crosslinking principle of SPDP is shown in Figure 1. Purchased McAb-C225 (1 mg) was mixed with SPDP (20 μg) for 30 min, then overdose SPDP was removed by dialysis in acetate buffer. DTT (15.4 mg) was added and the mixture was stirred for 30 min lightly. From the above procedure, McAb-C225 modified by sulfhydryl group was obtained. Appropriate siRNA-MANS was distributed in acetate buffer, SPDP (100 μL) was added. After 1 h of stirring at room
temperature, the reaction mixture (pshRNA-MANS-PDP) was centrifuged (12,000 rpm) and washed for three times. C225-SH and pshRNA-MANS-PDP were mixed and shaken for 24 h (4°C). The end-product (C225-pshRNA-MANS) through conjugation of disulfide bond was washed by purified water and freeze-dried. C225-siRNA-ANS (pshRNA-AINS) conjugates were prepared according to the same methods.

Characterization of MAINS

Nanoparticles suspension was added dropwise onto copper grids and dried at room temperature. Their morphological characteristics were observed by a TEM (JEM-2100 CX, Tokyo, Japan). Zeta PALS equipment (Brookhaven Instruments Corporation, Holtsville, NY) was carried out to determine the size and polydispersity index of the nanoparticles, which were dispersed in isotonic NaCl examined at 25°C with a scattering angle of 90°.

In vitro pshRNA-survivin release

Release of pshRNA from the nanospheres was carried out in acetate buffered solution (pH 7.2) as follows. pshRNA-MAINS were dispersed in 2 mL acetate buffered solution, and placed in a dialysis bag (molecular weight cut-off 8000 Da). The end-sealed dialysis bag was incubated in 100 mL acetate-buffered solution with gentle agitation. At predetermined time intervals, 2 mL of dialysis solution was withdrawn and replaced with an equal volume of fresh acetate-buffered solution. The concentration of pshRNA was determined through its optical densities tested by an ultraviolet spectrophotometer.

In vitro transfection experiment

The human lung cancer GLC-82 cell lines were cultured in DMEM medium with 10% fetal calf serum (Gibco, Grand Island, NY). Cells were grown at 37°C in a 5% (v/v) CO2 atmosphere.

GLC-82 cells were plated onto a six-well plate at a density of 3 x 10⁵ cells/ well overnight prior to the transfection experiment. The transfection was initiated by washing the cells twice with 1 mL of sterile phosphate-buffered solution (PBS) followed by incubation at 37°C with (a) blank ANs in 500 μL of serum-free culture media; (b) MAINs containing 4 μg of plasmid shRNA in 500 μL of serum-free media; (c) MANs containing 4 μg of pshRNA in 500 μL of serum-free media. Treatments were terminated after 6 h incubation and the cells were cultured in normal growth media for an additional 42 h. Then, the cells were observed by an inverted fluorescence microscopy.

Immunoreactivity of immuno-nanospheres

Slide agglutination test

The pshRNA-MAINs or pshRNA-MANs suspension was mixed with equivalent rabbit antimice IgG antiserum on the glass slide, which incubated for 30 min at 37°C. The agglutination between the nanospheres and antibody was observed by a light microscope.

Immunofluorescence analysis

The pshRNA-MAINs (immuno-nanospheres) were diluted in PBS buffer including serum, then rabbit antimouse IgG labeling with Alexa Fluor 488 was added the above buffer to incubate for 30 min (4°C). After washing with PBS buffer for three times, the samples were observed by a laser confocal scanning microscope (Olympus Fv1000, Tokyo, Japan). The control group was pshRNA-MANs. Then, the nanospheres labeled with or without Alexa Fluor 488 were used to incubate with the lung cancer GLC-82 cell lines fixed with cold acetone. Before combination, GLC-82 cells were cultured on the cover glass located in six-well plates in DMEM medium with 10% fetal calf serum (Gibco, Grand Island, NY) for 24 h. The incubated cells were washed three times with PBS, nucleus was stained by DAPI. Laser scanning confocal microscope was applied to observe the combination condition. The green excitation wavelength was 488 nm, blue was 358 nm.

Observation of immuno-nanospheres linking with cells

Scanning electron microscope observation

GLC-82 cells (3 x 10⁵ mL⁻¹ PBS containing serum) digested by Trypsinogen were mixed with pshRNA-MAINS for 30 min...
The sample was observed with a light microscope (Olympus CX21, Tokyo, Japan). The control groups were pshRNA-MANs and GLC-82 cells. After observation with a light microscope, the resulting mixture was stabilized in 2.5% glutaraldehyde for 30 min at 4°C, then dehydrated with 50%, 70%, 90%, 100% alcohol, every density lasting for 15 min and dried in air. The treated samples were observed with a scanning electron microscope (JEOL JSM-6360LV, Tokyo, Japan).

**Prussian blue staining**

Aliquots (1 mL) of GLC-82 cells (3 × 10⁵ mL⁻¹) in complete DMEM medium supplemented 10% fetal calf serum (Gibco) were seeded into 12-well plates, pshRNA-MAINs and pshRNA-MANs were added separately. After 24 h, each well was washed three times with PBS, treated with 0.5 mL of 4% paraformaldehyde for 30 min to fix the cells, and then washed with PBS. For staining of intracellular iron, 2 mL of Prussian blue solution, comprising equal volumes of 2% aqueous hydrochloric acid and 2% potassium ferrocyanide(II) trihydrate, was added to the fixed cells for 30 min. After washing with PBS, cells were observed under a microscope.

**The biological effects on lung adenocarcinoma in vitro**

**MTT assay**

The MTT assay was used to test inhibition of cell GLC-82 proliferation. GLC-82 cells in 96-well plate (6 × 10⁴ cells/well) were divided into five groups: (1) pshRNA-MAINs group; (2) pshRNA-AINs group; (3) pshRNA-MANs group; (4) C225 group; (5) positive control group. Each group contained six wells, groups (1) and (3) were treated with exterior magnetic field. After 48 h of incubation, cells proliferation was tested by the MTT assay. The optical densities (OD) of the samples were determined on a spectrophotometer at 490 nm. The inhibition rate (IR) of the cell proliferation = 1 – OD of the experimental group/OD of the control group.

**Flow cytometry**

GLC-82 cells were seeded into six-well plate (5 × 10⁵ cells/well), 37°C, 5% CO₂ culture for 24 h. The grouping was the same as MTT assay. After 48h of treatment, cells were collected for apoptosis analysis. Apoptosis ratio was measured by a flow cytometry.

**Statistical analysis**

Data are expressed as mean ± standard error of the mean analyzed by SAS statistical software (Chicago, IL). The statistical significance was performed using one-way ANOVA test. A value of p less than 0.05 was considered to be significant.

**Results**

**Characterization of Fe₃O₄ nanoparticles and albumin nanospheres**

The Fe₃O₄ nanoparticles were approximately spherical, uniform in size, and had good dispersibility with an average diameter of about 20 nm (Figure 2). The transmission electron micrographs of pshRNA-MAINs and ANs samples reveal morphological aspects of nanospheres with smooth surface and approximately spherical shape (Figure 3). Moreover, compared with the photograph of free-loaded albumin nanospheres (Figure 3a), the photograph of pshRNA-MAINs (Figure 3b) clearly displays that the magnetite Fe₃O₄ nanoparticles were well-incorporated in the matrix of albumin nanospheres. The prepared magnetic immuno-nanospheres size distribution is shown in Figure 4. The nanosphere size distribution was reflected by the polydispersity, which range from 0.0 for an entirely monodisperse sample to 1.0 for a polydisperse sample. Particle size analysis determined by dynamic light scattering reveals that the nanospheres had an average diameter of 206.6 ± 2.9 nm and the polydispersity index was 0.103.

**In vitro survivin-shRNA release**

The pshRNA-release curves in vitro for pshRNA-MAINs are depicted in Figure 5. Fast release (burst effect) occurred for 3 h after administration, then pshRNA was released slowly from the formation nanospheres. The slow release times were about 12 h. The rates of fast release in initial 3 h were 43%. The total content of pshRNA encapsulated (100%) was released within 24 h.

**In vitro transfection experiment**

The result of transfection experiment with nanospheres is presented in Figure 6. Light green fluorescence was observed in the cells of both pshRNA-MAINs group and pshRNA-MANs group, while no green fluorescence in the cells of blank ANs group. Furthermore, the result also reveals that the transfection efficiency of pshRNA-MAINs group is higher than the pshRNA-MANs group.

**Immunoreactivity of immuno-nanospheres**

**Slide agglutination test**

The result of slide agglutination experiment is shown in Figure 7. Slide agglutination test was used to detect whether
antibody binding with the nanosphere, the agglutination was clearly observed after MAINs incubated with antiserum compared with the control group (MANs), in which the albumin nanospheres were equably dispersed. These demonstrated that the antibody has bided with the nanosphere in MAINs group.

**Immunofluorescence analysis**

The graphics of laser confocal microscope show that the nanospheres modified monoclonal antibody were fluorescent (Figure 8a), while the nanospheres unmodified were negative (Figure 8b). Figure 9 shows the result of cell experiment, green fluorescence was observed on the surface of cell or in the cytoplasm in immuno-nanospheres group (Figure 9a). The distribution of the immuno-nanospheres was heterogeneous from one cell to another, while some cells were weakly labeled, the majority was successfully labeled. Besides, the repartition of the nanoparticles on the surface of the cells was irregular: some cells were homogeneously covered with particles and other presented unlabeled areas. Yet little green fluorescence was found in the control group (Figure 9b).

**Observation of immuno-nanospheres targeting to lung cells**

*Scanning electron microscope observation*

The pshRNA-MAINs and pshRNA-MANs were mixed with GLC-82 cells, respectively. As shown in scanning electron microscope images (Figure 10), immuno-nanospheres made a tight junction with GLC-82 cells and adhesion of pshRNA-MANs to the surface of GLC-82 cells was not observed. The photographs vividly show GLC-82 cells captured immuno-nanospheres or immuno-nanospheres caved in cells, while the surface of GLC-82 cell itself was near smooth.

*Prussian blue staining*

Prussian blue staining was used to observe intracellular iron oxide nanoparticles, the combination between nanospheres and cells can be directly observed by a light microscope.
The pictures (Figure 11) consistently showed dense intracellular iron labeling in these cells treated with pshRNA-MAINs composites, with iron exclusion from the nucleus. However, there is almost no obvious iron particles observed in the pshRNA-MANs group cells.

The biological effects on lung adenocarcinoma in vitro
The MTT assay and flow cytometry assay were performed to evaluate the biological effects of treated with the pshRNA-MAINs composites on human GLC-82 cells. Figure 12 shows the result of MTT assay, the cell growth inhibitory rates (IR) of pshRNA-MAINs group, pshRNA-AINs group, pshRNA-MANs group and C225 group were 65.95%, 51.58%, 39.58% and 27.52%, respectively, which were statistically different from the negative group (p < 0.05). So, the magnetic and monoclonal antibody targeting gene therapy group (pshRNA-MAINs group) could more significantly inhibit the proliferation of the GLC-82 cells compared to other therapy groups.

The results of flow cytometry assay are shown in Figure 13. All these experimental groups can induced the apoptosis of GLC-82 cells. The apoptotic indexes of the cells of pshRNA-MAINs group, pshRNA-AINs group, pshRNA-MANs group and C225 group were 24.80%, 17.18%, 7.6% and 7.84%, respectively, whereas the apoptotic index of the negative control group was 0.70%, which is identical to the result of MTT assay.

Discussion
The aim of this study was to prepare a double (magnetic field and antibody) targeting gene delivery system and to improve
Figure 7. The results of reaction between IgG antiserum and nanospheres. (a) pshRNA-MAINs and (b) pshRNA-MANs.

Figure 8. Fluorescence images of nanospheres after incubation with second antibody labeled fluorescence. (a) pshRNA-MAINs and (b) pshRNA-MANs.

Figure 9. Laser confocal photographs of GLC-82 cell treated with nanospheres. (a) pshRNA-MAINs; (b) pshRNA-MANs; (1) observed with the wavelength 378 nm and (2) with 488 nm (3) stack photo of 1 and 2.
the potential gene therapy effect. Inside the target cell the complexes should release the packaged DNA in a reasonable time course to supply sufficient and persistent plasmid concentrations that are able to access the nucleus, which may enhance the gene biological effect. We determined the curve of pshRNA release from the gene delivery system MAINs in vitro, which showed a release pattern characterized by an initial rapid release, followed by a slower release. The initial effect may be due to dissolution of pshRNA loaded near the surface of the nanospheres and the sustained release of phase could be due to the release of tightly entrapped plasmid, accompanied with matrix expansion and/or degradation. The characteristics of the transfer system just ensure the plasmid sustained release. The pictures of transfection show that pshRNA was successfully encapsulated in the matrix of nanospheres. We also observed that the transfection efficacy of pshRNA-MAINs is higher than that of pshRNA-MANs. Similar results have been published by others, Hayes et al. [25] studied an antiHER2-lipopolymer conjugated to a cationic lipid nanoparticle with the DNA encapsulated in its interior, which achieves a high degree of specific transfection activity. Because HSA NPs enter cells via caveolae or clathrin mediated pathway [5], the phenomenon may be attributed to the specific reorganization between antibody coated to the surface of nanoparticles and antigen on the tumor cell, which could increase the endocytosis to the immuno-nanospheres of tumor cells. This just offers a sufficient concentration in target cells.

The biological effect of many monoclonal anticancer antibodies including cetuximab is dependent on the cytotoxic response of the immune reaction induced against cancer cells coated with antigen-bound antibodies. Hence, the most important factors to be considered in the conjugation of monoclonal antibodies to cytotoxic drugs or the surface of nanoparticles concern the necessity of retaining the primary specific immunoreactivity of monoclonal antibodies. In the present study, immunoreactivity of immuno-nanospheres was assessed in vitro, the results of slide agglutination test and immunofluorescence analysis indicated that cetuximab mAb conjugated to the particles still maintains the feature of reacting with IgG antiserum. Moreover, the SEM photograph (Figure 10) visually shows that the MAINs were trapped by the lung cancer cell, the results of Prussian blue staining reveal abundant iron oxide particles in the cytoplasm of GLC-82 cell treated with immuno-nanospheres compared with non-targeted group. The above findings are indicative of the targeting capability of the immuno-nanospheres to GLC-82 cell, which can also be indirectly demonstrated through the results of immunofluorescence analysis. Many investigations have been carried out to evaluate targeting of the nanospheres coated with monoclonal antibodies. The methotrexate-loaded immunomicrospheres prepared by Lee et al. [26] showed selective affinity for the antigen-positive human leukemia cell line Daudi, yet non-specific binding of the antigen-negative cells was less than 3%. In the in vivo tests, Akasaka et al. have shown that BSA-NS coated with monoclonal antibodies could
be trapped in the tumoral tissue of Lewis lung carcinoma-bearing mice to a greater extent than in controls (BSA-NS coated with mouse IgG) at 24 h after the injection [27]. Hence, the enormous advantage of immuno-nanospheres compared with conventional therapy methods is that they are specific, thereby minimizing side effects, as they would be directed at their tumoral targets (antigens), without affecting or with only minimum cytotoxic on healthy tissues.

The First-Line Erbitux in Lung Cancer trials indicated that promising efficacy results of the combination with cetuximab significantly improved overall survival compared with chemotherapy alone [28,29] survivin-targeting siRNA have exhibited favorable therapy effect to lung cancer cell. Chen et al. [30] constructed a siRNA lentiviral vector targeting survivin gene, which efficiently and specifically down-regulated the expression of survivin at both the mRNA and protein levels, furthermore dramatically suppressed the proliferation of A549 lung cancer cells. The combination of siRNA-mediated gene-silencing strategy with chemobiological agents also acts to decrease tumor susceptibility to apoptosis and to increase angiogenesis and resistance to several anticancer drugs [31]. Hence, the combination of survivin shRNA and monoclonal antibody cetuximab may play a synergistic role and result in enhanced cell killing. The outcomes of MTT test and flow cytometry assay indicate that the pshRNA-MAINs with external magnetic field presents more effective in inhibiting proliferation of lung cancer cell and inducing apoptosis in cancer cells than other treatment methods. Analysis to the biological results among these treatment groups also shows that the cetuximab coated to MANs not just have the selective targeting to the expression EGFR cell, also could inhibit proliferation to lung cancer cell.
The above findings suggest that the novel pshRNA-loaded MAINs modified by cetuximab have specific targeting capability to the lung cancer cell GLC-82 and present excellent biological effect to lung cancer cell. However, there are still many works to be done, e.g. the molecular analysis of survivin gene expression and protein levels should be carried out in the next study and the characteristics and application of pshRNA-loaded MAINs in vivo still need to be investigated.

Conclusions

In this research, we have developed pshRNA-loaded magnetic immuno-nanospheres coinorporated with the plasmid survivin-siRNA and the superparamagnetic nanoparticles Fe3O4 by desolvation-crosslinking technique, and coated by the antiEGFR (cetuximab) monoclonal antibody. The nanospheres hold sustained and steady release of the plasmid. Furthermore, the monoclonal antibody conjugated still maintains the immuno-activity and render the nanospheres specific targeting to lung cancer cell. In addition to the guidance of external magnetic field, the nanospheres efficiently inhibited tumor cell growth and induced apoptosis. These advantages make the novel magnetic immuno-nanospheres a promising candidate for targeting therapy to lung cancer.

Declaration of interest

The authors would like to gratefully thank the Chinese National Natural Science Foundation (30770584 and 81171452). The authors report no conflicts of interest. The National Natural Science Foundation (30770584 and 2003CB515400) supported this work. The authors alone are responsible for the content and writing of this article.

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