Nanocontrast Agents For In Vivo Probing On Non-Small Cell Lung Cancer In MR Molecular Imaging

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Introduction

Molecular imaging of cancers provides valuable information regarding the clinical behavior of a disease and treatment response to certain therapeutic modalities. Among various molecular imaging modalities under development, magnetic resonance imaging (MRI) provides high spatial resolution, excellent perception with tomographic capabilities, outstanding soft-tissue contrast, and good anatomical detail and orientation. And nanosize materials can provide site-specific therapeutic action and minimize side effects in medicine.[1-3] Among this, magnetic nanoparticles play a significant role in MR study because of its superparamagnetic character.[4] In the previous study, the aqueous Fe3O4-NH2 nanoparticles show excellent negative contrast in MR images for in vitro and in vivo experiments due to reducing proton relaxation times of T1 and T2, and also been convinced its biocompatibility, hemocompatibility, and low cytotoxicity at in vitro tests.[5] Magnetic nanoparticles probe has already been a sharp weapon for tumor detection[6]. In this study, we choose non-small cell lung cancer as our probing target. As we know, lower than 15% patients with NSCLC survive longer than 5 years. In NSCLC, the EGFR is over-expression in 40-80% cases.

Materials and Methods

Tumor cell lines:
We choose three non-small cancer lung cell lines CL1-0, CL1-5, A549, and one monocyte THP-1. All of them were cultured with ATCC complete growth medium RPMI 1640 medium within 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 100U/ml penicillin G, 100g/ml streptomycin sulfate and 10% fetal bovine serum in a humidified atmosphere consisting of 5% CO2 in air at 37°C.

Monoclonal antibodies against the human EGFR EGFR Ab-10 (Clone 111.6) mouse Mabs that immuno-react with human EGFRs was purchased from Becton Dickinson Co., USA; and 2% HCl. At least, use 1/1000 dilution of the monoclonal antibody for tumor experiments. We then evaluated the molecular imaging of cancer lesions for small lung cancer cells and monocyte. In vitro experiments, it shows a significant targeting difference between NSCLC and monocyte. And in vivo MR molecular imaging, the Fe3O4-anti-EGFR antibody nanoparticles also exhibited the negative imaging contrast enhancement in tumor regions in 6 hr.

Results and Discussions

After measuring the FITC fluorescence emission events in 10000 cells of each cell lines with flow cytometry, NSCLC cell CL1-5 and A549 were presented a very high level. The next is NSCLC cells CL1-0. THP-1 shows almost background level of FITC emission. On the other hand, result of iron stain is presented a very high level. The modification of Fe3O4-NH2 nanoparticles was used the traditional chemical cross-linking method. The iron oxide nanoparticle was reacted with antibody to form a covalent bond by catalyzing with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and the molar ratio of Fe3O4-NH2 nanoparticles and anti-EGFR antibody was 1 : 5[5].

In vivo MR Imaging:
To evaluate the MR enhancement efficiency of Fe3O4-anti-EGFR antibody nanoparticles, in vivo MR images were performed in the SCID (mice) tumor model. The mice were anesthetized using isoflurane and then given Fe3O4-anti-EGFR antibody nanoparticles (10.0 mg/kg), dispersed in normal saline, and injected via the jugular vein with a 30G needle connected to a syringe with 100 cm polylethylene tubing. The MR experiment data were acquired using a 3T Medspec/Biospec MRI system (Bruker, Ettlingen, Germany) with an inserted mini-gradient system; i.d. was 12 cm and the maximum gradient strength was 200 mT/m. An actively decoupled RF transmit coil with an inner diameter of 7.2 cm (Bruker BioSpin) was used for RF transmission, and a receive-only surface coil, including low noise preamps (RAPID Biomedical), was used for RF reception. All of imaging processing and quantitative measurement of tissue signal intensity was used Matlab software (version 7.0.1. The MathWorks, Inc.) and ImageJ (Image) 1.41, National Institutes of Health, USA; http://rsb.info.nih.gov/ij/java 1.5.1_13)

Perl’s iron stain:
The other way to directly perceive the expression of EGFR in NSCLC cells is Perl’s iron stain. Original cultured cells were harvested with versene (EDTA) solution. Using 24 well cell culture microplates to subculture cells. There are 5x106 cells every well. Fe3O4-NH2-antibody nanoparticles were added to incubate about 2 hrs. Perl’s iron stain procedure was performed in both nanoparticles-added group and control group. Add 4% buffered paraformaldehyde for fixing cells and then incubate with Perl’s working solution containing 1:1 ratio of 2% potassium ferrocyanide and 2% HCl. At last, use nuclear fast red to stain nucleus.

Conclusions

We have demonstrated that Fe3O4-NH2 nanoparticles conjugated with anti-EGFR antibody are capable of probing NSCLC cells in vitro and in vivo. Furthermore, its large different expression between NSCLC cells and monocyte provide nanoparticles higher chance to target the extracellular domain of epidermal growth factor receptor in non-small cell lung cancer cells and monocyte. And we also have investigated the biodistribution, and kinetics of the nanoparticles. By recombining various functional nanoparticles through bioconjugation, this modularly designed platform has the capability of enhancing the efficiency of targeted diagnosis and therapies for a wide spectrum of biomedical applications.

References