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 characteristic[4]. In the previous study, the aqueous Fe_3O_4-NH_3^+ nanoparticles show excellent negative contrast in MR images for *in vitro* and *in vivo* experiments due to reducing proton relaxation times of T_1 and T_2, and also been convinced its biocompatibility, hemocompatibility, and low cytotoxicity at *in vitro* tests[5]. Magnetic nanoparticles MR 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. Over-expression of EGFR in NSCLC is correlated with a high metastatic rate, poor tumor differentiation, and a high rate of tumor growth[7,8]. This is already convinced depend on histology examination. To make use of this phenomenon, we use Fe_3O_4-NH_3^+ nanoparticles coated with antibodies to target the extracellular domain of epidermal growth factor receptor in non-small cell 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 Fe_3O_4@anti-EGFR antibody nanoparticles also exhibited the negative imaging contrast enhancement in tumor regions in 6 hr.

Materials and Methods

Tumor cell lines We chosen three non-small cell lung cancer 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 sodium, 100μg/ml streptomycin sulfate and 10% fetal bovine serum in a humidified atmosphere consisting of 5% CO_2 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 *LAB VISION / NEOMARKERS*. It will target to the extracellular domain of EGFR and show no cross-reaction with cerbB-2, c-erbB-3, or c-erbB-4.

Magnetic resonance nanoparticles coated with anti-EGFR Antibody The modification of Fe_3O_4-NH_3^+ nanoparticles was used the traditional chemical cross-linking method. The iron oxide nanoparticle were reacted with antibody to form a covalent bond by catalyzing with 1-ethyl-3-(3-dimerthylaminopropyl)-car-bodiimide (EDC), and the molar ratio of Fe_3O_4-NH_3^+ nanoparticles and anti-EGFR antibody was 1 : 5[5].

In vivo MR imaging To evaluate the MR enhancement efficiency of Fe_3O_4@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 Fe_3O_4@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 polyethylene 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 maximal gradient strength was 200 mT m^{-1}. An actively decoupled volume 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 (ImageJ 1.41j, 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 5×10^4 cells every well. Fe_3O_4-NH_2-antibody nanoparticles were added to incubate about 2 hrs. Perls' iron stain procedure was performed in both nanoparticles-added group and control group. Add 4% buffered paraformaladehyde for fixing cells and then incubate with Perls' working solution containing 1:1 ratio of 2% potassium ferrocyanide and 2% HCl. At lest, use nuclear fast red to stain nucleus.

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 coincidence with result in flow cytometry. The Fe_3O_4-NH^{3+} nanoparticles patched on the cell surface by the antibody targeting shows blue color after Perl's stain. It shows that EGFR are over-expression in CL1-5 and A549, and also expressed a high level in CL1-0. However, to confirm the error estimation from cytophagic nanoparticles, electron microscope scanning should be supplied. We then evaluated the molecular imaging of cancer lesions for specific membrane protein in lung cancer SCID mice animal model. For the MRI of each tumor lesion, we injected Fe_3O_4@anti-EGFR antibody nanoparticles at a concentration of 10 mg/kg through the right jugular vein and then sequentially acquired images at 1, 2, 3, and 6 h intervals in a 3T MR imager. Fe_3O_4@anti-EGFR antibody nanoparticles yielded a specifically targeted negative contrast image of the oral cancer lesion within 6 h using a T2* pulse sequence (Figure 1a). The signal intensity of the tumor region was decreased by nanoparticles targeting (~25% reduction of signal intensity at 4 hr after injection (Figure 1b).

Conclusions

We have demonstrated that Fe_3O_4-NH_3^+ nanoparticles conjugated with anti-EGFR antibody were 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 EGF receptors in tumor cells. And we also have investigated the biodistribution, and kinetics of the nanoparticles. By recombining the desired targeting moiety and 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.

a) b)

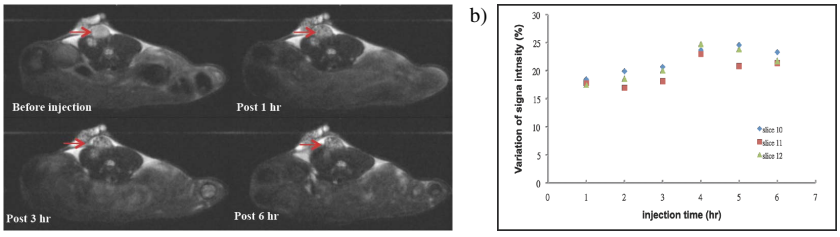

Figure 1. a) *In vivo* molecular magnetic resonance imaging of A549 lung cancer in SCID mice model shows significant inverse contrast T_2* images 1, 3, and 6 h after injection of the Fe_3O_4@anti-EGFR antibody nanoparticles. (The red arrow areas indicate the tumor lesion). b) Quantitative measurement (*Matlab* software) of tumor signal intensity showed that modifying the magnetite nanoparticles with the tumor targeting that were significantly enhanced their signal intensity in the tumor region.

References

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