Multi-functional Nanocontrast Agents for *In Vivo* Probing on Non-Small Cell Lung Cancer in MR and Optical Molecular Imaging

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Introduction

Molecular imaging has become an indispensable technology in cancer research and clinical use. Among various molecular imaging modalities, magnetic resonance imaging (MRI) provides high spatial resolution and soft-tissue contrast in global view and optical microscopy supplies excellent time and spatial resolution in local view [1-3]. In the previous study, nano-sized Fe_3O_4 particles showed significantly negative contrast in the MR imaging, and nano-sized quantum dot revealed high fluorescence signals [4-5]. Multi-functional nanocontrast agents including Fe_3O_4 and quantum dot are supposed to provide the two contrast effect in the imaging. Therefore, the goal of this study is to combine magnetic resonance imaging and optical imaging system with multifunctional contrast agent to detect xenograft non-small cell lung cancer (NSCLC) murine model.

Materials and Methods

Tumor cell lines Two non-small cell lung cancer cell lines CL1-0 and A549 were chose and 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.
Nanoparticle preparation High temperature solution phase reaction led to 8 nm ultrasmall superparamagnetic iron oxide (USPIO, Fe_3O_4), and the CdS-capped $CdTe_xSe_{1-x}$ alloyed quantum dot was synthesized to near-IR emitting nanoparticles.
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.
Multi-functional nanoparticles coated with anti-EGFR Antibody The hydrophobic magnetic resonance nanocontrast, Fe_3O_4 and optical nanocontrast, quantum dot were modified to hydrophilic and joined by chemical cross-linking method. The ration of Fe_3O_4-Lysine and CdS-capped $CdTe_xSe_{1-x}$-NH_3^+ nanoparticles was 1:2 and the linking reaction catalyzed by 1-ethyl-3-(3-dimerthylaminopropyl)-car-bodiimide (EDC). The multi(bi)-functional nanoparticles were reacted with antibody to form a covalent bond by catalyzing with EDC, and the molar ratio of nanoparticles and anti-EGFR antibody was 1 : 5.
In vitro fluorescence microscope imaging The imaging was captured by microscope (Olympus BX51) with 1230 nm femtosecond Cr:forsterite laser.
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 (5.0 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 7-Tesla MRI system (BioSpec 70/30 USR, Bruker) with a 12 cm inner bore diameter gradient (BGA12-S, Bruker) ,and a linear transmitter coil (T10720V3, Bruker) for RF transmission and array coil (T7399V3, Bruker) 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.).
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

In the *in vitro* imaging experiments, Prussian blue staining imaging and fluorescence microscope imaging (Figure 1.) showed different targeting efficiency in A549 and CL1-0. In the *in vivo* imaging, T2 and T2* MR imaging showed more than 30% signal decrease (Figure 2. a) T2 imaging). And the change of the signal intensity in the two adjacent slices reveals similar tendency in the time intervals from 0 to 5 hours after injection.(Figure 2. b) It was proved the dynamic contrast change caused by nano-probe targeting by using histological cytochemistry staining.

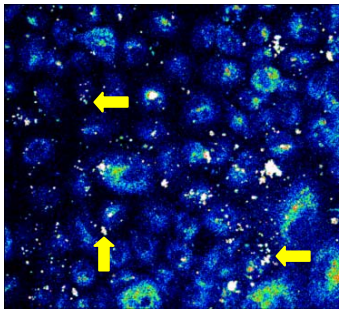

Figure 1. *In vitro* fluorescence microscope imaging of CL1-0 lung cancer cells with multi-functional nanocontrast. (Indicated by the arrow)

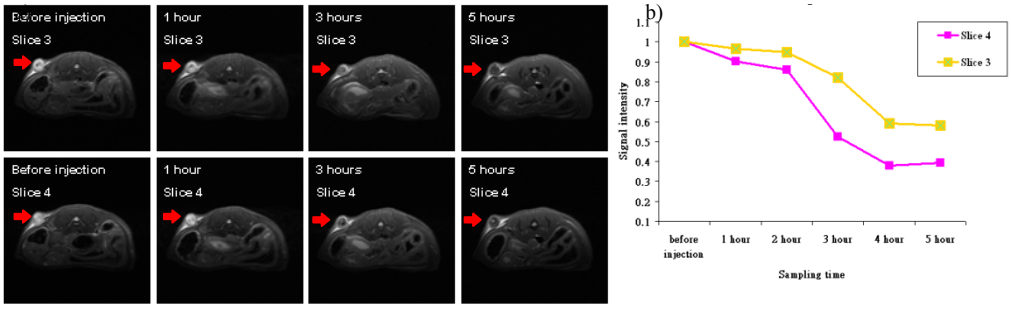

Figure 2. a) *In vivo* molecular magnetic resonance imaging of CL1-0 lung cancer in SCID mice model shows significant negative contrast T_2 images 1, 3, and 5 h after injection of multi-functional contrast coated anti-EGFR antibody. (The red arrow areas indicate the tumor lesion). b) Quantitative measurement 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.

Conclusions

We have demonstrated that multi-functional contrasts including Fe_3O_4 and quantum dot conjugated with anti-EGFR antibody were capable of probing NSCLC cells *in vitro* and *in vivo*. And we also have investigated the biodistribution, and cytotoxicity of the nanoparticles. Multifunctional nanocontrast agent could hopefully not only serves as cancer detection and treatment but also used to predict disease prognosis in the future. 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.

References

[1] T. N. Lambert, N. L. Andrews, H. Gerung, T. J. Boyle, J. M. Oliver, B. S. Wilson, S. M. Han, *Small*, **2007**, *3*, 691. [2] J.-H. Lee, Y.-M. Huh, Y.-w. Jun, J.-w. Seo, J.-t. Jang, H.-T. Song, S. Kim, E.-J. Cho, H.-G. Yoon, J.-S. Suh, J. Cheon, *Nat. Med.* **2007**, *13*, 95. [3] D. Peer, J. M. Karp, S. Hong, O. C. Farokhazd, R. Margalit, R. Langer, *Nat. Nanotech.* **2007**, *2*, 751. [4] A. K. Gupta, and M. Gupta, *Biomaterials*, **2005**, *26*, 3995-4021. [5] I.L. Medintz, H. Mattoussi, A.R. Clapp, *Int J Nanomedicine*. **2008**; 3(2), 151.