Magnetic Resonance Fe_3O_4-NH_3^+ Nanoparticles Coated with Anti-Epidermal Growth Factor Receptor Antibody for *in vitro* probing on Non-Small Cell Lung Cancer

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Introduction

Following the higher magnetic field, better resolution, and higher SNR, magnetic resonance molecular imaging is becoming a powerful mechanism on researches and medical diagnosis. Among this, magnetic nanoparticles play a significant role in MR study because of its superparamagnetic characteristic[1]. 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 T1 and T2, and also been convinced its biocompatibility, hemocompatibility, and low cytotoxicity at *in vitro* tests[2]. Magnetic nanoparticles MR probe has already been a sharp weapon for tumor detection[3]. 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[4,5]. 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.

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,1.5g/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[2].

Flow cytometry : To make sure there are large amount of EGF receptors over-expressed on NSCLC cells membrane, we used goat-anti-mouse antibody conjugated with FITC to target EGFR antibody. By utilizing Flow cytometry *FC500* (*Beckman Coulter*), the quantities of FITC of 5×10^5 cell density in every cell lines would be well measured to present the expression of EGFR. In order to eliminate background noise and non-specific binding of goat-anti-mouse antibody, we have setup three sets of negative control included (1)without EGFR antibody and FITC goat-anti-mouse antibody, (2)with EGFR antibody only,(3)with FITC goat-anti-mouse antibody only in each cell line.

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. Use 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 (Figure 1c) and A549 (Figure 1e), and also expressed a high level in CL1-0 (Figure 1a). However, to confirm the error estimation from cytophagic nanoparticles, electron microscope scanning should be supplied.

Conclusions

We have demonstrated that Fe_3O_4-NH_3^+ nanoparticles conjugated with anti-EGFR antibody were capable of probing NSCLC cells *in vitro*. 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. Base on these characteristics, we could use Fe_3O_4-NH_3^+nanoparticles as the MR targeting probe in the followed *in vitro* and *in vivo* proton relaxation time measurements in 3T Bruker MRI to detect NSCLC and the disease model of lung cancer.

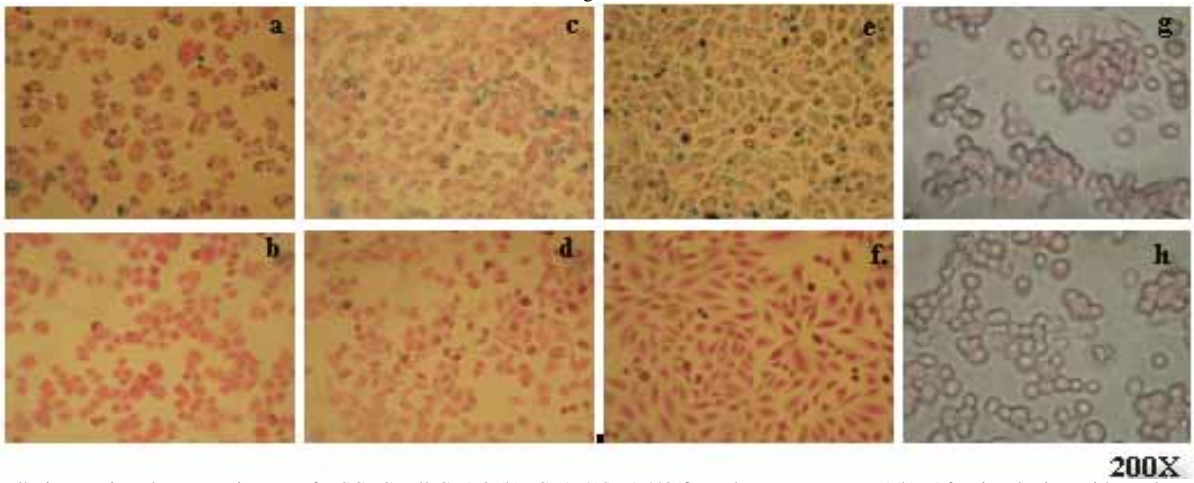

Figure 1: Perl's iron stain. There are pictures of NSCLC cell CL1-0 (b), CL1-5(d), A549(f), and monocyte THP-1(h). After incubation with Fe_3O_4-NH_2-antibody nanoparticles 2 hours, the results of stain are CL1-0(a), CL1-5(c), A549(e), THP-1(g) respectively.

References

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