The characteristics of human antibody targeting the Epidermal Growth Factor Receptor in vivo for radioimmunotherapy in a small animal model

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1. Introduction

The identification of epidermal growth factor receptor (EGFR) as an oncogene has led to the development of anticancer therapeutics directed against EGFR, including Erbitux for colon cancer. Many therapeutic approaches are aimed at the EGFR. Erbitux is example of monoclonal antibody inhibitors. The monoclonal antibodies block the extracellular ligand binding domain. EGFR4-2, IgG human monoclonal antibody, has been developed on the basis of human antibody gene library in Green Cross Corp. Small animal imaging is useful for preclinical evaluation of radiolabeled antibody to see biodistribution and targeting ability at serial time points in same animals.

2. Methods and Results

2.1. *Preparation of radioiodine labeled antibodies*

Antibodies were radioiodinated using the Iodo-Bead reagent. A single Iodo-Bead was washed with 0.5 ㎖ of 0.2 ㏖/ℓ phosphate buffer (pH 6.5) and dried on filter paper. Cleaned Iodo-bead was added to an eppendorf tube. 1 mCi of radioiodine and equal volume of 0.2 ㏖ /ℓ pre-incubation at room temperature (25℃), 200 ㎍ of antibodies was added. After 30 minutes incubation at room temperature (25℃), the reaction is terminated by removing Iodo-Bead. Radiolabeling yield was determined by radio thin layer chromatography. TLC plate was silica coated glass and developing solution was acetone.

2.2. Cell line and Cell culture

Human epidermoid carcinoma A-431 cell was obtained from Laboratory of Antibody Engineering in Green Cross Corp. A-431 was maintained as monolayer cultures in DMEM medium (Gibco, USA) supplemented with 10% fetal bovine serum, 100 units/ ㎖ penicillin and 100 ㎍ / ㎖ streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37℃.

2.3. In vitro binding test

All steps in the antibodies binding assay were done at 4℃. Cells were washed 3 times with D-PBS (PBS pH 7.4 without $CaCl_2$ and $MgCl_2$). Cell numbers were counted and incubated for 1 hour with probe ^{125}I labeled antibodies without or with constant concentration cold antibodies. Afterwards, cells were washed twice with casein blocker. Radioistope was counted in a 1480 automatic gamma counter (Wallac, Finland). To correct for radioactive decay, standards were counted simultaneously. The 50 % cell specific binding number of [I^{125}]Erbitux in EGFR overexpressing A-431 was found to be 5.3 X 10^4 cells, whereas [I^{125}]EGFR4-2 in A-431 was 2.4 X 10^5 cells. The binding affinity of Erbitux was better than EGFR4-2.

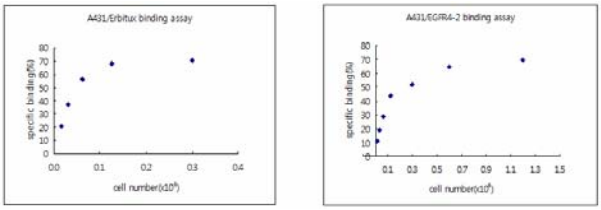

Fig. 1. A-431 cell line EGFR binding assay.

2.4. Experimental animal models

6-week-old female athymic BALB/c-nu Slc nude mice (Charles river, Japan) were housed five/cage and provide sterilized water. Tumor cells were harvested near confluence by incubation with 0.02% trypsin-EDTA. Cells were pelleted by centrifugation at 1,500 rpm for 3 minutes and resuspended in serum free media. Cells (2~3 X 10^6/animal) were implanted subcutaneous into the front right thigh of mice. Animal were carried for approximately 2 weeks until tumors reached roughly 1 ㎝ in size.

2.5. Gamma camera images of radiolabeled antibodies in small animal models

To obtain gamma camera image, radio-labeled antibodies were injected into tail vein. Mouse was anesthetized with 2% isoflurane and placed in posterior views at the microSPECT (Inveon; Siemens, Erlangen, Germany). Each image was acquired for 2 mm pin-hole collimator was used. In the xenograft model, accumulation of [I^{123}]Erbitux and [I^{123}]EGFR4-2 was examined by microSPECT. In planar images of [I^{123}]Erbitux and [I^{123}]EGFR4-2, A-431 tumor region

accumulation of [I^{123}]Erbitux and [I^{123}]EGFR4-2 increased as elapsed time.

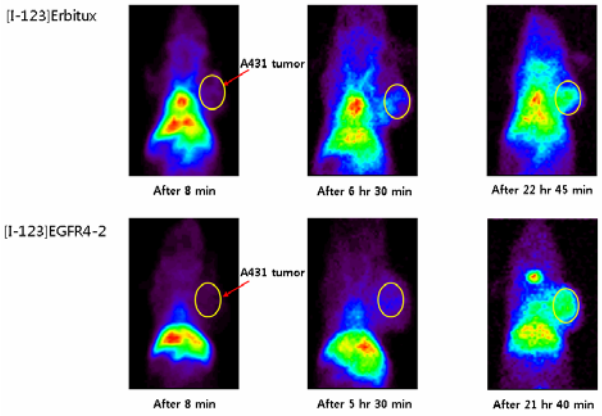

Fig. 2. Gamma camera images of athymic mice bearing A-431 tumors using 4.07 MBq of ^{123}I-labeled Erbitux and EGFR4-2.

3. Conclusions

This study indicates that EGFR 4-2 antibody is feasible for EGFR expressed tumor targeting. Imaging result offers better assessments of pre-clinical studies, and improves evaluations of antibodies evaluation. EGFR4-2 was found to specifically bind to its receptor. EGFR4-2 has an advantage that is human antibody.

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