Radioiodination of Antibody protein using FCCS12026, a novel linker for increasing stability against deiodination in vivo

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1. Introduction

Radioiodine is most commonly employed to prepare radiolabeled protein with high specific activity for *in vitro* and *in vivo* applications[1,2]. However, a major shortcoming of radioiodinated proteins prepared by direct labeling methods is deiodination *in vivo*. To decrease deiodination, a new bifunctional linker for radioiodination of proteins, N-(4-Isothiocyanatobenzyl)-2-(3-(tributylstannyl)phenyl)acetamide (FCCS12026), was designed and synthesized[3]. The aims of this study are to optimize conditions for radioiodination of an antibody and to assessment of immunoreactivity by Lindmo assay[4].

2. Methods and Results

2.1 Indirect radiolabeling of Cetuximab using the linker

For the indirect radiolabeling, FCCS12026 was radioiodinated using chloramine-T to give, N-(4-Isothiocyanato benzyl)-2-(3-[^{125}I]phenyl)acetamide ([^{125}I]-FCCS12027) which was purified by radio-HPLC. To optimize conditions for radioiodination of cetuximab, the mixture of [^{125}I]-FCCS12027 and Cetuximab was incubated at various condition. Cetuximab (200 µg; 5.0 mg/ml) in carbonate buffer (pH 9.4) or borate buffer (ph 8.5) was added to ^{125}I-labled FCCS 12026 in DMSO. The mixture was vortexed and incubated at Room temperature, 4 or 37 °C for 1, 2, 4 or 24 h. Then the radiolabeling yield was measured by radio-TLC (n=3).

Table 1: Radiolabeling yield after 1, 2, 4 or 24 h reaction of [^{125}I]-FCCS 12027 with Cetuximab in borate buffer at various temperature

(unit : %)

temperature	1h	2h	4h	24h
4°C	22.57 ±0.82	25.84 ±2.52	28.81 ±3.47	46.85 ±2.84
Room temperature	45.45 ±5.61	58.98 ±0.79	73.47 ±2.93	84.33 ±0.78
37°C	80.16 ±1.92	84.86 ±2.10	91.20 ±0.77	37.53 ±2.95

Table 2 : Radiolabeling yield after 1, 2, 4 or 24 h reaction of [^{125}I]-FCCS 12027 with Cetuximab in Cabonate buffer at various temperature

(unit : %)

temperature	1h	2h	4h	24h
4°C	32.07 ±1.01	33.60 ±2.03	24.17 ±0.14	62.54 ±1.63
Room temperature	55.39 ±1.90	67.44 ±1.05	75.83 ±1.66	76.18 ±1.63
37°C	80.63 ±1.21	38.46 ±2.06	30.40 ±2.17	38.16 ±1.30

Radiolabeling yield of [^{125}I]-FCCS12027-Cetuximab was influenced by buffer PH, reaction time and incubation temperature. In conclusion, the optimized condition for [^{125}I]-FCCS 12027-Cetuximab is as in the following. The reaction buffer, time and temperature were borate buffer, 2h and 37°C. Labeled mAb was purified by Zeba™ Spin Desalting column, 0.5ml (Thermo scientific, Piscataway, NJ, USA) using DPBS as running buffer. Then radiochemical purities were measured by radio-TLC.

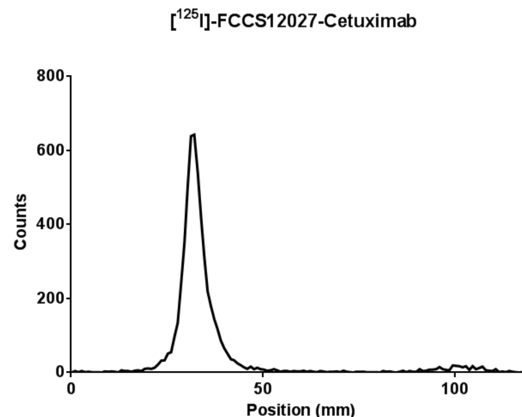

Fig. 1. Result of radio-TLC about reaction of [^{125}I]-FCCS 12027 with Cetuximab at 37°C for 2h in Borate buffer.

2.2 cell binding assay

[^{125}I]-FCCS12027-Cetuximab was prepared by optimized condition as described above. Direct labeling antibody, [^{125}I]-Cetuximab is prepared by chloramine-T method. Cetuximab (60 µg; 5.0 mg/ml) in BupH Phosphate buffer (pH 7.2) were added Na^{125}I in 0.1N NaOH, followed by 10 µl of a 1 mg/ml solution of Chloramine T in BupH phosphate buffer. After 20 sec at

room temperature, the reaction was terminated with 10μl of a 2.5mg/ml solution of sodium metabisulfite. The labeled monoclonal antibodies were isolated by Zeba^{TM} Spin Desalting column, 0.5 ml (Thermo scientific, Piscataway, NJ, USA) using DPBS as running buffer. Then radiochemical purities were measured with radio-TLC.

PC 9 cells (human lung adenocarcinoma) were grown in RPMI, supplemented with 10% fetal bovine serum (FBS; JHR Biosciences, Lenexa, KS), and 1% antibiotics (Gibco, Carlsbad, CA). The medium was changed twice or three times per week. The cells were cultured at 37 °C in a 5% CO_2 atmosphere.

Serial dilutions of PC 9 cells (1×10^7–2×10^4 cells) in 1% BSA/PBS were incubated with [^{125}I]-Cetuximab and [^{125}I]-FCCS12027-Cetuximab (20 kcp m) for 1 h at 4 °C. The cells were then centrifuged and washed twice with 1% BSA/PBS[5]. The radioactivity of the pellet was counted with gamma counter (Wizar d; PerkinElmer). The immunoreactivities of radiolabel led antibodies were estimated by Lindmo method[5].

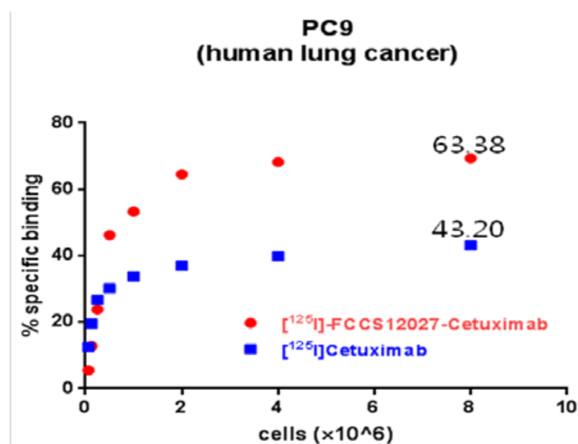

Fig. 2. cell binding assay of [^{125}I]-Cetuximab and [^{125}I]-FCCS 12027-Cetuximab to PC-9 cells

3. Conclusions

We have optimized reaction conditions for [^{125}I]-FCCS 12027-Cetuximab. This immunoreactivity result supports that newly developed FCCS12027 will be a promising bifunctional linker for radioiodination of proteins for *in vivo* applications including radioimmuno -imaging and therapy

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