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-Isothiocyanatobenzyl)-2-(3-[125I]phenyl)acetamide ([125I]-FCCS12027) which was purified by radio-HPLC. To optimize conditions for radioiodination of cetuximab, the mixture of [125I]-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 [125I]-labeled 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 [125I]-FCCS 12027 with Cetuximab in Cabonate buffer at various temperature (unit : %)

<table>
<thead>
<tr>
<th>temperature</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>22.57 ± 0.82</td>
<td>25.84 ± 2.03</td>
<td>28.81 ± 3.47</td>
<td>46.85 ± 2.84</td>
</tr>
<tr>
<td>Room temperature</td>
<td>45.45 ± 5.61</td>
<td>55.39 ± 1.05</td>
<td>73.47 ± 1.66</td>
<td>76.18 ± 1.63</td>
</tr>
<tr>
<td>37°C</td>
<td>80.63 ± 1.21</td>
<td>84.86 ± 2.06</td>
<td>30.40 ± 2.17</td>
<td>38.16 ± 1.30</td>
</tr>
</tbody>
</table>

Radiolabeling yield of [125I]-FCCS12027-Cetuximab was influenced by buffer PH, reaction time and incubation temperature. In conclusion, the optimized condition for [125I]-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.

![Radio-TLC](image)

2.2 cell binding assay

[125I]-FCCS12027-Cetuximab was prepared by optimized condition as described above. Direct labeling antibody, [125I]-Cetuximab is prepared by chloramine-T method. Cetuximab (60 μg; 5.0 mg/ml) in BupH Phosphate buffer (pH 7.2) were added Na125I 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™ 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% CO₂ atmosphere.

Serial dilutions of PC 9 cells (1×10⁷–2×10⁴ cells) in 1% BSA/PBS were incubated with [¹²⁵I]-Cetuximab and [¹²⁵I]-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 (Wizald; PerkinElmer). The immunoreactivities of radiolabeled antibodies were estimated by Lindmo method[5].

3. Conclusions

We have optimized reaction conditions for [¹²⁵I]-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.

REFERENCES