1. Introduction

Recently, in the field of nuclear medicine, research on radioimmunotherapy (RIT) is being actively conducted. RIT uses a radioisotope as a marker to administer an antibody that specifically reacts with a tumor-targeting antigen to the patient to identify the tumor site, and radiotherapy is performed to that area.

Epidermal growth factor receptor (EGFR) is a key biomarker used for cancer diagnostics and therapy in non-small cell lung cancer (NSCLC). Genetic mutation of the EGFR occurs in approximately 40-55% of patients with NSCLC in East Asians [1,2]. Cetuximab is a recombinant chimeric anti-EGFR IgG1 monoclonal antibody that competitively binds to the EGFR extracellular domain and blocks receptor activation by growth factor [3]. It has been investigated in advanced colorectal cancer, EGFR-expressing NSCLC, and unresectable squamous cell skin cancer [4].

Tumor suppression and therapeutic effects using therapeutic isotopes have been proven through various studies, and it is well-known that therapeutic isotopes are useful for cancer treatment. However, the exact mechanism of action of cancer treatment by therapeutic radioisotopes on the molecular level has not yet been elucidated.

In this study, we observe the synergistic effect of cancer treatment as well as changes in the mechanism of action on the molecular level by conjugated with therapeutic radioisotopes to Cetuximab, a well-studied and well-known EGFR tumor-targeting antibody. Therefore, it could be used as basic data for the development of various therapeutic radioisotopes in the future by specifically identifying the molecular mechanism of known radioimmunotherapy drugs.

2. Methods and Results

2.1 Analysis of EGFR mRNA expression in NSCLC cell line

The mRNA expression level of EGFR and cell apoptosis related gene (RUNX3, TP53) in NSCLC cell line were analyzed using data from Cancer Cell Line Encyclopedia (CCLE). NSCLC human cancer cell line were selected for EGFR expression based on CCLE databases.

As shown in Fig.1, H520 (lung squamous cell carcinoma) cell line does not express EGFR, it was used negative control. The other two cell lines, H1975 and HCC827 (lung adenocarcinoma), are over-expressed by EGFR mutations. These cell lines were used in this study for validation of therapeutic radioisotope.

2.2 Analysis of EGFR protein expression in NSCLC cell line

H520, H1975 and HCC827 human cancer cell lines were obtained from the American Type Culture Collection (ATCC) and cultured in RPMI1640 supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. All cell lines were incubated at 37°C with 5% CO2.

Cells were lysed with RIPA lysis buffer, and the lysates were quantified. The cell lysates were separated using SDS-PAGE and transferred to PVDF membranes. The proteins were identified using the individual antibodies, and the protein signal was detected using an enhanced chemiluminescence kit.

Fig. 1) Heat map illustrating differential mRNA expression level in NSCLC cell line

Fig. 2) Western blot analysis for EGFR signaling (phospho-EGFR, MAPK, phospho-MAPK, AKT, phospho-AKT) and cell apoptosis related (p53, RUNX3) protein expression
As shown in Fig.2, the EGFR and cell apoptosis related (RUNX3, p53) protein expression pattern similar to the mRNA expression obtained from the CCLE databases. The NSCLC cell lines except for the negative control cell line, H520, overexpressed EGFR by EGFR mutations. Also, the RUNX3-p53 axis is known to be a gene involved in apoptosis, but the expression of RUNX3 is very low and p53 overexpression by TP53 gene mutation suppresses apoptosis in NSCLC cell lines. The abnormal expression of the tumor suppressor genes RUNX3 and p53 is associated with cancer progression in NSCLC cells.

2.3 Effect of Cetuximab on tumor cell growth

Each cell line was transferred into a 96-well plate [5x10^5 cells per well (H520, HCC827) and 3x10^5 cells per well (H1975)] and incubated at 37°C overnight. The cells were treated in triplicate with different concentrations of Cetuximab. After 48h treatment at 37°C, WST (water soluble tetrazolium salt) solution was added to each well and the reaction was allowed to proceed for 1h at 37°C. Absorbance of each well was read at 450nm and directly correlated to number of remaining viable cells. Absorbance data were normalized to percentage of vehicle-treated control, then graphed.

As shown in Fig.3, in the cell line with high EGFR expression (HCC827) the effect of Cetuximab was significantly higher than that of the cell line with low EGFR expression (H1975). This shows a correlation between the high expression of EGFR and the cell growth inhibitory effect of Cetuximab.

2.4 Effect of Cetuximab on tumor cell death

Each cell line was transferred into a 6-well plate [5x10^5 cells per well (H520, HCC827) and 3x10^5 cells per well (H1975)] and incubated at 37°C overnight. The cells were treated with 10µg/ml of Cetuximab. After 24h treatment at 37°C, Cells were lysed with RIPA lysis buffer, and the lysates were quantified. The cell lysates were separated using SDS-PAGE and transferred to PVDF membranes. The proteins were identified using the individual antibodies, and the protein signal was detected using an enhanced chemiluminescence kit.

As shown in Fig.4, treatment with Cetuximab caused down-regulation of total EGFR and EGFR phosphorylation in EGFR-overexpressing cell line. The MAPK pathways activity (MAPK phosphorylation), one of the downstream signaling pathways of EGFR signaling, was slightly down-regulation. Also, Cetuximab induced PARP cleavage, a marker of cell death. In conclusion, Cetuximab inhibited EGFR expression and induced tumor cell death.

Fig. 3) Inhibition of NSCLC cell proliferation by Cetuximab

Fig. 4) Cetuximab inhibits EGFR activation and induced tumor cell death

3. Conclusions and Future works

Cetuximab down-regulated EGFR tumor-targeting antigens and induced cell growth inhibition and cell death of EGFR-overexpressing tumor cell. These results suggest that Cetuximab has therapeutic efficacy and can function as effective carriers for tumor-targeted delivery of radioisotope in EGFR-overexpressing NSCLC cells. Through this study, cells suitable for the purpose were selected and conditions were prepared for the confirmation of tumor cell death by Cetuximab. Later, we plan to conjugate Cetuximab with a potential therapeutic radioisotope ([^7]Scandium, [^177]Lutetium) to search for the optimal conditions for tumor cell death and to determine the mechanism of action of therapeutic radioisotopes. In addition, since abnormal expression of the tumor suppressor genes RUNX3 and p53 induces the progression of NSCLC cell line, the role and mechanism of action of RUNX3 and p53 in the induction of apoptosis by Cetuximab and therapeutic radioisotopes will be confirmed.

In conclusion, this study aims to verify the usefulness and therapeutic efficacy of therapeutic radioisotopes, and elucidates the mechanism of antitumor effect of therapeutic radioisotopes on the molecular level.
REFERENCES