学位論文内容の要旨

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学 位 論 文 題 名

Development of a novel cancer therapy with a ferroptosis inducer and radiation, and a prediction method for therapeutic effects targeting ferroptosis
（フェロトーシス誘導剤と放射線を併用した新規がん治療法、及びフェロトーシス誘導がん治療効果予測法の開発）

Background and Objectives

Drug resistance in cancer chemotherapy is a critical issue in clinical practice, and a novel therapeutic strategy is desired to overcome this problem. Recently, ferroptosis, a novel programmed cell death triggered by iron-dependent accumulation of lipid peroxides, was identified and taken an interest. Iron homeostasis is often disrupted in cancer cells, which leads to excessive accumulation of iron compared to normal cells. Therefore, therapeutic effects of cancer ferroptosis-targeting strategy are being expected because of its iron dependency. In this study, I aimed to establish a precision medicine system for a novel effective cancer therapeutic strategy targeting ferroptosis.

A small molecule anticancer drug erastin was first discovered as an inducer of ferroptosis triggered by cystine glutamate antiporter (xCT) inhibition, which leads to a loss of antioxidant capacity. Antioxidants capacity of cancer cells plays a critical role in cancer resistance to radiotherapy and chemotherapy. Hence, erastin treatment is expected to enhance the efficacy of radiation treatment against cancer cells. Therefore, the first chapter aimed to develop a novel cancer therapy strategy combining a ferroptosis inducer and X-irradiation.

Previous research has demonstrated the anticancer effect on several ferroptosis inducers and suggested that the efficacy of the ferroptosis-targeting cancer therapy could be different between the cancer types. To avoid unnecessary or ineffective health care, development of a technique for predicting ferroptosis sensitivity in cancer is required. In chapter 2, I aimed to establish a prediction method of the therapeutic effect of ferroptosis-targeting cancer therapy by a positron emission tomography (PET) probe targeting transferrin receptor 1 (TfR1), a key molecule for cellular iron homeostasis and ferroptosis.

Chapter 1

Materials and Methods

The clonogenic ability, glutathione peroxidase 4 (GPX4) expression, glutathione concentration, and iron concentration were evaluated using HeLa and NCI-H1975 adenocarcinoma cell lines treated with erastin and/or X-ray irradiation. Expression levels of GPX4 and TfR1 in the cancers were evaluated by western blot analysis. Intracellular and intratumoral glutathione concentrations were evaluated by colorimetric method. Intracellular iron concentrations were measured by multiple inductively coupled plasma atomic emission spectrometry (ICP-AES). For in vivo studies, NCI-H1975 cells were transplanted in the left shoulder of nude mice, and then radiosensitizing effect of erastin and glutathione concentration in the cancers were evaluated.

Results

Erastin treatment induced ferroptosis and decreased the GPX4 protein expression levels and concentration of glutathione in the two different cancer cell lines. Although, the erastin treatment did not affect intracellular iron concentration and TfR1 protein expression level in neither HeLa and NCI-H1975 cells. Erastin enhanced X-ray irradiation-induced cell death in both the cell lines. Erastin treatment of a tumor-transplanted mouse model similarly demonstrated the radiosensitizing effect and decrease in intracancer glutathione concentration to the in vitro study.

Discussion

In this study, I demonstrated the radiosensitizing effect of erastin on two adenocarcinoma cell lines and the tumor xenograft model accompanied by glutathione depletion, indicating that drugs inducing ferroptosis with glutathione depletion may have a potential application as a novel cancer therapy in combination with radiotherapy.

Chapter 2

Materials and Methods

Human renal cancer cell lines, A498 and 786-O cells, were used in this study. p-isothiocyanatobenzyl-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and human-apo-transferrin (aTf) were conjugated to form NOTA-aTf and radiolabeled with ^{68}Ga eluted from a generator. Radiolabeled ^{68}Ga-NOTA-aTf was reacted with excess amount of ferric citrate in order to bind iron with aTf to form iron bound holo-transferrin (hTf). The number of NOTA conjugated to aTf was examined by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and the concentration of transferrin-bound iron was measured by ICP-AES. Expression levels of TfR1 in the cancers were evaluated by western blot analysis. The sensitivity of the cancers to erastin in vitro were evaluated by a clonogenic assay. Lastly, an uptake of the synthesized ^{68}Ga-NOTA-aTf/hTf by the cancer cell lines were evaluated.

Results

The TfR1 expression level of 786-O cells was relatively higher than A498 cells. The erastin sensitivity of 786-O cells was significantly higher than A498 cells. The cellular uptake of ^{68}Ga-NOTA-hTf was significantly higher in 786-O cells compared to A498 cells (1.0% and 0.6% of total activity, respectively).

Discussion

The expression levels of TfR1 in human renal cancer cell lines were correlated to erastin sensitivity. Moreover, the cellular uptake of ^{68}Ga-NOTA-hTf reflected the TfR1 protein expression levels of the cancer cells. Thus, this study demonstrated the potential of ^{68}Ga-NOTA-hTf to predict therapeutic effects of ferroptosis-targeting cancer therapy. Predicting erastin sensitivity of cancer cells by ^{68}Ga-NOTA-hTf PET imaging before the treatment may motivate to select ferroptosis-targeting treatment.

Summary and Conclusion

In this study, I succeeded to develop a novel cancer therapeutic strategy focused on ferroptosis inducer. My study suggested that the ferroptosis-targeting cancer therapy could be an alternative therapeutic strategy for the cancer patients suffering from a drug resistance. Moreover, I succeeded to develop a prediction method of ferroptosis sensitivity with PET tracer for the first time in the world. By using this method, it will make possible to provide best therapy approach to the individual patients. Taken together, these studies have contributed as a first step toward the development of precision medicine system.