（様式４）

学 位 論 文 の 内 容 の 要 旨

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（学位論文のタイトル）

^{64}Cu-ATSM Predicts Efficacy of Carbon Ion Radiotherapy Associated with Cellular Antioxidant Capacity

（^{64}Cu-ATSMは細胞の抗酸化能力に関連する炭素イオン放射線療法の有効性を予期する）

（「論文目録（様式３）」の主論文の部分を記載する。英文の場合は和訳をつける。）

（学位論文の要旨）2,000字程度、Ａ４判

Purpose: Carbon ion radiotherapy is an emerging cancer treatment modality that has a greater therapeutic window than conventional photon radiotherapy. To maximize the efficacy of this extremely scarce medical resource, it is important to identify predictive biomarkers of higher carbon ion relative biological effectiveness (RBE) over photons. We addressed this issue by focusing on cellular antioxidant capacity and investigated 64Cu(II)-diacetyl-bis(N4-methylthiosemicarbazone) (64Cu-ATSM), a potential radioligand that reflects an over-reduced intracellular environment.

Methods and Materials: In vitro and in vivo sensitivities of human cancer cell lines to X-rays or carbon ions were assessed by clonogenic assays and mice xenograft growth delay assays, respectively. In vitro and in vivo ^{64}Cu-ATSM uptake was assessed by radioactivity assays and a small animal PET, respectively. Cellular redox state was assessed by immunoblotting, flow cytometry, and liquid chromatography coupled to tandem mass spectrometry.

Results and discussion: The cell lines showed variable sensitivities to the radiation; with greater cell killing for carbon ions than for X-rays in all cell lines examined. RBE values ranged from 1.3 +/- 0.15 (for low RBE HCT15)) to 2.8 +/- 0.48 (for high RBE, H1299), with a median of 2.0; consistent with clinical beam set-up of CIRT, for reference HSG cell line. This indicates that wider therapeutic window, are anticipated for tumors showing RBE values greater than that of HSG cells (> 2), therefore such tumors should be stratified preferentially for CIRT. The relative antitumor effect of carbon ions to X-rays observed in the xenograft model was consistent with in vitro RBE, suggesting the robustness of the xenograft models as a means of in vivo validation. Cellular ^{64}Cu-ATSM uptake by the cancer cell lines under normoxic conditions in vitro showed peak uptake at 30 min and cell were saturated at 60 min post-treatment, which is consistent with previous publications. Carbon-ion RBE showed strong a correlation with ^{64}Cu-ATSM uptake measured at 30 mins of exposure. Similarly, ^{64}Cu-ATSM uptake by H1299 xenografts was significantly greater than that by HCT15 xenografts. The SUVmax for ^{64}Cu-ATSM uptake predicted the cell line for xenografts significantly. These data indicate that high RBE tumors can be identified by PET imaging using 64Cu-ATSM, a finding that warrants

clinical validation. Taken together, carbon-ion RBE correlated with ^{64}Cu-ATSM uptake both in vitro and in vivo. Recent studies highlight another emerging biological property of 64Cu-ATSM, that it represents an intracellular over-reduced state. Hence, we conducted series of redox experiment to evaluate redox status of cancer cells, which demonstrated that high RBE/^{64}Cu-ATSM cells showed greater steady-state levels of antioxidant proteins and increased capacity to scavenge reactive oxygen species in response to X-rays than low RBE/^{64}Cu-ATSM counterparts. In addition, we found that the high RBE/^{64}Cu-ATSM uptake status of cancer cells were associated with the downregulation of TCA cycle intermediates These findings highlight cellular antioxidant activity is a potential determinant of carbon-ion RBE and TCA cycle activity as a surrogate biomarker of high RBE/^{64}Cu-ATSM uptake. Since metabolites assessment by LC-MS less expensive than PET imaging, this option should be further explored. Furthermore, inhibition of nuclear factor erythroid 2-related factor 2 (Nrf2) (a cellular antioxidant system regulator) by Brusatol sensitized high RBE/^{64}Cu-ATSM cells to X-rays, thereby reducing RBE values to levels comparable to those in low RBE/^{64}Cu-ATSM cells. Brusatol did not sensitize tumors to carbon ions, irrespective of the RBE and ^{64}Cu-ATSM uptake. This is consistent with the understanding that direct ionization is the predominant mode of DSB induction by carbon ions. More importantly, in the presence of brusatol, RBE values for high and low RBE/^{64}Cu-ATSM cells were comparably low (i.e., <2.0). These data suggest that the RBE of carbon ions is dependent largely on the cellular capacity to mitigate the indirect effect of X-rays, whereas the cell killing effect of carbon ions against various cancers is more consistent than that of X-rays, supporting the rationale that photon-resistant tumors should be treated with CIRT.

The study has the following limitations. First, we did not use LET-specific carbon-ion beams. This was because we intended to mimic the clinical situation by using SOBP beams that have mixed LET profiles. Second, in the LC-MS analysis, we did not analyze the metabolites other than the six TCA cycle intermediates due to technical difficulties. A more comprehensive analysis, taking these factors into account, will pro-vide more detailed mechanistic insight into the association between carbon ion RBE, antioxidant activity, and ^{64}Cu-ATSM uptake by cancer cells.

Conclusion: In the present study, we show for the first time that the RBE of carbon ions is associated with ^{64}Cu-ATSM uptake and with antioxidant capacity in cancer cells. These new findings highlight the potential utility of ^{64}Cu-ATSM imaging to identify high RBE tumors that will benefit from CIRT.