Title: Radio-selective effects of a muscle-derived dipeptide in an external beam treated non-small cell lung cancer (NSCLC) mouse model

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Abstract

Introduction: Combining radio-sensitizing agents with External Beam Radiotherapy (RT) improves radiation-mediated tumor cell killing. The ideal radio-sensitizer should improve RT-mediated tumour cell inactivation by affecting cancer cells only. The dipeptide L-Carnosine (CAR) shows promise, having radio-sensitizing and anti-neoplastic properties *in vitro*, and is clinically safe. We seek to validate CAR as an *in vivo* radio-sensitizer which improves radiation-mediated cancer cell inactivation while reducing radiation-mediated healthy tissue damage.

Methods: Clonogenic assays were conducted to determine the effect of combining varying radiation doses and CAR concentrations *in vitro*. To evaluate *in vivo* effects, nude athymic mice were surgically implanted with H1299 in an orthotopic lung tumour model. When tumors reached 5-10 mm^3 animals were randomly divided into four groups: 1) Control, 2) RT-only, 3) CAR-only and 4) CAR+RT. Control and RT-only received 8-days of intraperitoneal vehicle, while CAR-only and CAR+RT received 8-days of daily 500 uL of intraperitoneal 1 M CAR. After 4-days of injections, RT treatment delivering a single 20 Gy dose were planned and delivered for RT-only and CAR+RT animals. Bi-weekly computed tomography imaging was conducted for 30-days post-RT to track tumour progression. Animals were sacrificed after 30-days or when they reached the humane endpoint. Lungs were collected and processed for analysis.

Results: Pre-treatment of H1299 with 30 mM CAR (CAR+RT) *in vitro* significantly reduced the surviving fraction by 30% when compared to RT-only, with the reduction increasing beyond a dose of 2.5 Gy. CAR+RT treated animals had significantly smaller tumors by 86% after 11-days post-RT and showed significantly reduced expression of the proliferation marker ki67. CAR+RT animals survived longer compared to all groups. Lung tissue surrounding the tumor showed reduced macrophage numbers for CAR+RT treated animals, when compared to CAR-only or RT-only.

Conclusion: CAR could be an effective radio-sensitizer, as it significantly reduced the proliferation of tumor cells and overall tumor size, in CAR+RT treated animals therefore improving tumour control.