

B-4

Loss of MEN1 Function Inhibits DNA Repair Capability of Pancreatic Neuroendocrine Tumors after Radiation Exposure

Xavier M. Keutgen, Olga Lakiza, Julian Lutze, Alyx Vogle, Jelani Williams, Andy Liao, Sean Pitroda, Carlos Martinez, Andrea Olivas, Namrata Setia, Stephen J. Kron, Ralph Weichselbaum
University of Chicago Medicine, Chicago, IL

BACKGROUND: Somatic MEN1 mutations occur in up to 50% of pancreatic neuroendocrine tumors (PanNETs). Radiation therapy (IR) is effective in a subset of PanNETs. Herein, we study whether MEN1 loss of function increases radiosensitivity and determine its effect on DNA double strand break (DSB) repair.

METHODS: A MEN1 knockout cell line (MEN1-KO-QGP1) was generated using CRISPR-Cas9. Clonogenic assays were performed and athymic nude mice xenograft models were created using wild type and MEN1-KO-QGP1 clones. Neutral Comet assays and immunofluorescence (IF) co-staining were used to assess DNA damage response. Cytotoxicity of combining IR with poly (ADP-ribose) polymerase 1 (PARP1) inhibitors was evaluated with clonogenic assays.

RESULTS: Both in vitro clonogenic assays and in vivo mice xenograft models showed significantly increased radiosensitivity of the MEN1-KO-QGP1 clone when compared to wild type QGP1 cells ($p < 0.01$ and $p < 0.0001$). Transient downregulation of MEN1 using siRNA confirmed these findings in 3 PanNET cell lines (QGP1, BON-1 and INS-1). Comet assays showed an increase in DNA double strand breaks (DSB) at baseline, 4 and 24 hours after IR. IF yielded significant variations in γ -H2AX, Rad51 and 53BP1 levels in MEN1-KO-QGP1 cells at different time points following IR, suggesting a decrease in DNA DSB repair and the homologous recombination (HR) pathway. MEN1 loss of function led to a decrease in BRCA2 mRNA/protein expression levels in MEN1-KO-QGP1 and in MEN1 siRNA-treated QGP1 cells. Lastly, PARP1 inhibition (10nM Talazoparib) significantly increased radiotoxicity in MEN1-KO-QGP1 cells when compared to

wild type QGP1 cells, particularly at lower IR doses ($p < 0.01$ to < 0.001).

CONCLUSION: MEN1 loss of function sensitizes PanNET cells to IR and influences DNA repair pathway choice by lowering BRCA2 expression and inhibiting HR. Combining IR with PARP inhibition may be beneficial in PanNET patients with somatic MEN1 mutations.

ABSTRACT ID: 132