Postradiation Sensitization of the Histone Deacetylase Inhibitor Valproic Acid

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Purpose: Preclinical studies evaluating histone deacetylase (HDAC) inhibitor-induced radiosensitization have largely focused on the preirradiation setting based on the assumption that enhanced radiosensitivity was mediated by changes in gene expression. Our previous investigations identified maximal radiosensitization when cells were exposed to HDAC inhibitors in both the preirradiation and postradiation setting. We now expand on these studies to determine whether postirradiation exposure alone affects radiosensitivity.

Experimental Design: The effects of the HDAC inhibitor valproic acid (VA) on postirradiation sensitivity in human glioma cell lines were evaluated using a clonogenic assay, exposing cells to VA
up to 24 h after irradiation. DNA damage repair was evaluated using γH2AX and 53BP1 foci and cell cycle phase distribution was analyzed by flow cytometry. Western blot of acetylated γH2AX was done following histone extraction on AUT gels.

**Results:** VA enhanced radiosensitivity when delivered up to 24 h after irradiation. Cells accumulated in G$_2$-M following irradiation, although they returned to baseline at 24 h, mitigating the role of cell cycle redistribution in postirradiation sensitization by VA. At 12 h after irradiation, significant γH2AX and 53BP1 foci dispersal was shown in the control, although cells exposed to VA after irradiation maintained foci expression. VA alone had no effect on the acetylation or phosphorylation of H2AX, although it did acetylate radiation-induced γH2AX.

**Conclusions:** These results indicate that VA enhances radiosensitivity at times up to 24 h after irradiation, which has direct clinical application.