Role of PI3K/Akt Signaling in TRAIL and Radiation-induced Gastrointestinal (GI) Apoptosis

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Background
- Activation of the PI3K/Akt pathway leads to tumorigenesis, progression, and resistance to therapy; inhibition of the PI3K/Akt pathway has been shown to radiosensitize tumor cells in vitro and in animal models.
- The results of activation or inhibition of this pathway on normal tissue response to radiation (RT) are not well characterized.
- This study was designed to determine the effects of physiologic and genetic manipulation of Akt signaling on RT-induced GI apoptosis in mice.

Materials and Methods
- Activation or inhibition of the PI3K/Akt pathway was studied in both cell lines and in mice.
- Insulin, a potent stimulator of PI3K/Akt, was used to activate the pathway in vitro and in vivo.
- Akt1 knockout mice were utilized to study inactivation of the pathway in vivo.
- The role of the PI3K/Akt pathway in affecting pure apoptotic death in cell lines was determined by adding insulin and glucose (for pathway activation) to TRAIL (TNF-alpha Related Apoptosis Inducing Ligand); activation of the pathway was measured via Western blotting for phospho-Akt (P-Akt).
- Mice were injected intraperitoneally with insulin and glucose, and stimulation of the PI3K/Akt pathway in vivo was measured via P-Akt (S473) immunofluorescence.
- To determine the effects of PI3K/Akt pathway activation on RT-induced apoptosis, mice were given a single fraction of 5 Gy 30 minutes after receiving intraperitoneal insulin and glucose.
- To determine the effects of Akt depletion on RT-induced apoptosis, Akt1 null and wild-type mice were given a single fraction of 5 Gy.
- GI tissues were harvested 6 hours, 1 day, and 4 days following RT, and apoptosis was measured via TUNEL immunofluorescence.

Results
- Insulin resulted in transiently increased levels of P-Akt levels, with a decrease after several hours in both cell lines and in the mouse colon; P-Akt levels were maximally elevated 30-90 minutes after injection.
- Treatment with insulin and glucose appeared to protect cell lines from TRAIL-induced apoptosis.
- RT caused apoptosis in the mouse ileum and colon at 6 hours, but induced minimal apoptosis in the liver or esophagus.
- Pre-treatment with insulin and glucose did not appear to protect against RT-induced apoptosis in the mouse ileum or colon.
- RT led to increased P-Akt levels 6 hours after treatment.
- RT caused increased apoptosis in the small intestinal crypt cells and colon cells of Akt1 null versus wild-type mice.

Author's Conclusions
- Activation of the PI3K/Akt pathway may contribute to therapeutic resistance.
- Stimulation of Akt with insulin can protect cells from TRAIL-induced apoptosis.
- Although additional stimulation of Akt by insulin did not protect against RT-induced apoptosis, RT alone caused...
increased levels of Akt, possibly secondary to an inherent adaptive response

- Knockout of Akt-1 sensitized cells to RT-induced apoptosis in the gut
- Although inhibition of the P13K/Akt pathway may increase sensitivity to both TRAIL- and RT-induced apoptosis, it may also result in increased GI toxicity

**Clinical/Scientific Implications**

This pre-clinical study was able to demonstrate modulation of radiation effects on normal tissues via manipulation of the PI3K/Akt signaling pathway in animal models. Additionally, resistance to TRAIL-induced apoptosis in vitro was increased by activation of the pathway. Though PI3K/Akt modulation may prove to be a promising mechanism for altering cell response to TRAIL or RT, response of normal tissues may also be altered, potentially decreasing any gains in the therapeutic ratio. These data also raise the interesting issue of the role of deranged Akt signaling, such as in diabetes and RT toxicity.