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Inhibition of hedgehog signaling by cyclopamine prodrug: targeted therapy for advance prostate cancer

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The Hedgehog (Hh) signalling pathway specifies patterns of cell growth and differentiation during embryogenesis in a wide range of tissues, including the prostate. In particular, advanced, metastatic prostate cancers demonstrate striking pathway activation, and inhibition of Hh signaling by the smoothened antagonist, cyclopamine leads to profound growth inhibition in vitro, and prolongs survival in mice bearing metastatic prostate cancers. Thus, targeted inhibition of Hh signaling may have significant implications of prostate cancer therapeutics. Cyclopamine is a potent and specific inhibitor of Hh signaling. However, the effects of systemically administered cyclopamine are not restricted to cancer cells. The lipophilic nature of this small molecule mandates its distribution in cell membranes, including passage across the blood brain barrier. Secondly, the requirement for Hh signaling in *normal* somatic cells implies that cyclopamine may be associated with potential systemic toxicities, precluding human administration of this promising anti-cancer agent. Herein, a prostate cancer-specific targeting strategy is outlined that will overcome these limitations of cyclopamine. To achieve targeted cytotoxicity, this small molecule is converted to biologically inactive prodrugs by coupling to a peptide carrier or peptide linked with a spacer group. These inactive prodrugs can be efficiently converted back to active Hh inhibiting agents only upon proteolysis by the serine protease activity of a unique prostate-specific protein, Prostate-Specific Antigen (PSA). PSA specific cleavage map of semenogelin I and II generated series of fluorescently tagged peptides that were assayed for PSA-specific hydrolysis. From these studies two peptides with the sequence His-Ser-Ser-Lys-Leu-Gln (HSSKLQ) and SSKYQ were identified for further evaluation because of specific and efficient PSA hydrolysis. We synthesized cyclopamine-prodrugs i.e. MuHSSKLQ-Cyclopamine and MuSSKYQ-Cyclopamine. Enzymatic hydrolysis assay of the prodrugs MuSSKYQ-Cyclopamine and MuHSSKLQ-Cyclopamine with PSA indicated 50% release of active drug cyclopamine over a period of 12−22 h. The MuSSKYQ-Cyclopamine prodrug appears to be efficiently hydrolyzed by PSA. The progress of the release of active parent drug (cyclopamine) and the peptide MuHSSKLQ-OH were determined by HPLC. Studies are now in progress in order to evaluate the efficacies of prodrugs against human prostate cancer model.

Radiation interactive agents

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Hypoxia assessed in malignant gliomas with [F-18]-fluoromisonidazole (FMISO) PET before and after radiotherapy (RT)

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Background: Hypoxia is associated with resistance to RT and chemotherapy in malignant tumors including gliomas. Due to the extremely low retention in normal brain, FMISO is an effective quantitative imaging agent for hypoxia in brain tumors. If higher burden of hypoxic tumor predicts poorer treatment results, then better therapies aimed at eliminating hypoxia need to be developed. We report our experience measuring hypoxia in glioblastoma multiforme (GM) with FMISO PET before and after RT to assess the relationships between hypoxic volume or maximum FMISO uptake and the time to tumor progression (TTP) and survival.
Materials and Methods: Nineteen patients were studied between their diagnostic surgery and the beginning of RT. Thirteen were studied after RT. Each had a 20 min scan 2 hours after iv injection of 7.0 mCi of FMISO. Regions of interest over tumor and normal brain areas were constructed on co-registered MRI T1+Gd images applied to the PET images. Venous blood samples taken during imaging were used to create tissue to blood concentration (T/B) ratios. T/B values above 1.2 were used to determine the hypoxic volume (HV) for each patient's tumor and brain regions (Rajendran, Eur J Nucl Med Mol Imaging, 2003). Maximum T/B values (T/Bmax) and T/C (tumor/cerebellum) (T/Cmax) were determined from the pixel with the highest uptake. TTP and survival were calculated from the date of surgery. Progression was defined by MRI criteria.
Results: Kaplan-Meier plots demonstrated shorter TTP and survival in patients whose tumors before RT contained hypoxic volumes, tumor T/Bmax or T/Cmax ratios greater than the median ($p < 0.05$). In regression analyses, greater hypoxic volume, tumor T/Bmax or T/Cmax were associated with shorter TTP and survival ($p < 0.05$). The data after RT did not reach significance.
Conclusions: These results suggest that greater burden of hypoxic GM prior to but not after RT predicts poorer TTP and survival. These findings could simply mean that greater tumor volume per se predicts worse TTP and survival since more volume likely leaves more tumor at risk for becoming hypoxic. This interpretation may apply to the hypoxic volume results but not necessarily to the tumor T/Bmax or T/Cmax results since these indicators of hypoxia could be independent of tumor volume. MRI T1Gd and T2 tumor volumes need to be compared to the hypoxic volumes in further analyses.
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c-MET inhibition radiosensitizes melanoma by inhibiting double strand DNA repair

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Purpose/Objective(s): Melanoma has proven relatively resistant to current cytotoxic therapies, while multiple mechanisms undoubtedly contribute to this process, the ability to effectively repair sublethal damage appears to be of particular importance. Signal transduction via the receptor tyrosine kinase c-Met has been shown, to confer protection from cytotoxic response triggered by DNA damage. Thus inhibiting c-Met may radiosensitize resistant tumor types such as melanoma. The small molecule inhibitor HPK-56 was designed in our lab to inhibit cKit and PDGF. When evaluated against a panel of protein kinases it exhibits strong inhibition of c-Met, at nanomolar concentrations.
Materials and Methods: The ability of HPK-56 to enhance the response to radiation induced sublethal damage was examined using the human melanoma cell line SB-CL2. Cell viability following treatment was demonstrated using an MTS assay. Double strand breaks were visualized by confocal microscopy and quantitated by FACS analysis following staining of gammaH2AX. pAKT levels were demonstrated by western blot analysis. Affymetrix microarrays were used to evaluate the gene expression profile of treated cells.
Results: The results of MTS assays indicate that HPK-56 alone produced cell death with a IC-50 of 5 *u*M level. When combined with radiation cell kill was enhanced by approximately one log, compared to radiation alone. Using gamma H2AX to detect and quantify double strand breaks, pretreatment with 5 uM HPK-56 was shown to produce a 5 fold increase in DNA damage 8hrs after XRT, compared to XRT alone.

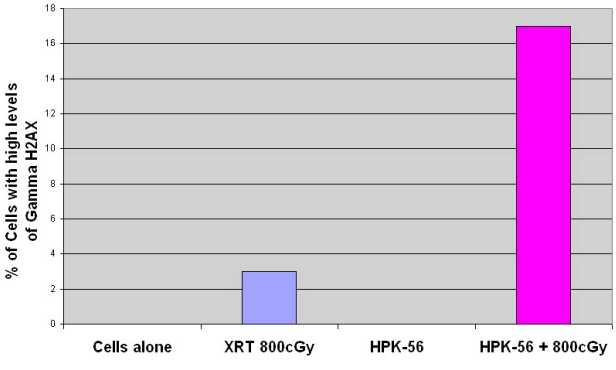

Fig. 1. Double strand DNA breaks in SB-CL2 melanoma cells.

The potential molecular basis of this activity was examined by western blot analysis demonstrating that treatment of SB-CL2 cells with HPK-56 reduced expression of pAKT. Microarray analysis revealed that HPK-56 inhibited several cell cycle/DNA repair genes such as K-ras2 (16 fold), ATM (14 fold), XRCC3-(X Ray repair) (12 fold) along with several oncogenes.
Conclusions: c-Met is a pro survival gene providing resistance to various cytotoxic therapies and implicated in a variety of human malignancies. A small molecular inhibitor designed in our lab has been shown to inhibit the tyrosine kinase activity of c-Met. We demonstrated that this inhibitor radiosensitizes a human melanoma cell line and increases double strand breaks at 8hrs after XRT by 5 fold. This response appears to be mediated by inhibition of several key regulators of the cell cycle and DNA repair, such as pAKT, K-ras2 and ATM. These findings suggest that c-Met inhibition leads to reduced DNA repair following XRT and when translated