530 POSTER

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

S.R. Khan, S. Kumar, R. Gong, A. Miatra, P. Beachy. *Johns Hopkins University, Oncology, Baltimore, USA*

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

531 POSTER

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

A. Spence[1], M. Muzi[2], K. Swanson[3], J. Rockhill[4], J. Rajendran[2], T. Adamsen[2], J. Link[2], J. Scharnhorst[1], K. Krohn[1]. *[1]University of Washington School of Medicine, Neurology, Seattle, WA, USA; [2]University of Washington School of Medicine, Radiology, Seattle, WA, USA; [3]University of Washington School of Medicine, Pathology, Seattle, WA, USA; [4]University of Washington School of Medicine, Radiation Oncology, Seattle, WA, USA*

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.
Supported by NIH grants Nos. PO1 CA42045 and S10 RR17229.

532 POSTER

c-MET inhibition radiosensitizes melanoma by inhibiting double strand DNA repair

J. Welsh, D. Mahadevan, G. Dougherty, B. Stea. *University of Arizona, Radiation Oncology, Tucson, USA*

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.

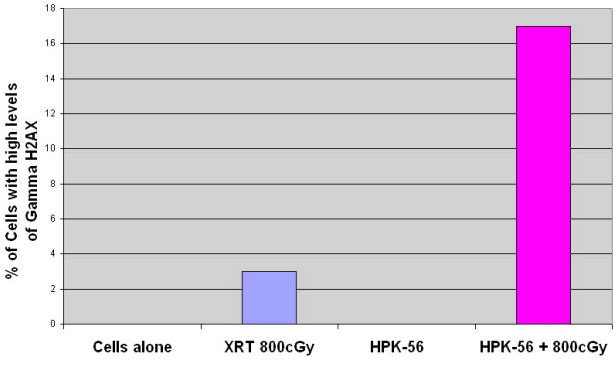

Fig. 1. Double strand DNA breaks in SB-CL2 melanoma cells.

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

successfully into a clinical setting could potentially help to improve local control and subsequently influence survival.

533 POSTER

RTA 401 (CDDO) and RTA 402 (CDDO-Me), promising new anti-cancer agents that also prevent oral mucositis

C. Meyer[1], D. Ferguson[1], B. Watkins[2], E. Fey[2], S. Sonis[3]. [1]*Reata Pharmaceuticals, Inc., Dallas, USA;* [2]*Biomodels, LLC, Cambridge, USA;* [3]*Brigham and Women's Hospital, Boston, USA*

RTA 401 and RTA 402 are novel synthetic triterpenoids that have demonstrated potent anti-cancer and anti-inflammatory activities. Both are in phase 1 clinical trials as anti-cancer agents. We have previously reported that these agents have significant radioprotective activity in a model of oral mucositis induced by acute radiation. This activity results from induction of the phase 2 response via the Keap1/Nrf2/ARE pathway. To further assess the anti-mucositis activity of these compounds in additional clinical settings, we studied RTA 401 and 402 in preclinical models of oral mucositis induced by chemo-radiation-induced and by fractionated radiation. Two studies were conducted in the Golden Syrian Hamster. In the chemo-radiation model, animals received 60 mg/kg of 5-FU IP on Day (D) -4 and D-2 followed by 30 Gy of radiation directed to the left buccal cheek pouch mucosa on D0. The rest of the animal was protected with a lead shield. In both studies, RTA 401 was administered at 5 mg/kg IP BID from D-9 to D15 or D-5 to D-1. RTA 402 was administered *per os* at either 6.5 or 9 mg/kg BID from D-9 to D15. Groups of 8–10 animals were used. Animals were evaluated clinically for mucositis from D6 through D28. Mucositis was scored visually by comparison to a validated photographic scale ranging from 0 for normal to 5 for severe ulceration. Ulceration was defined as a score of $\geqslant 3$. The primary endpoint was the number of days with mucositis scores of $\geqslant 3$ (D3+). Chi-Squared analysis was performed to assess the significance of the difference in D3+ between treated and control groups. Both RTA 401 and RTA 402 demonstrated significant anti-mucositis activity in each study. In the chemo-radiation study, the best results were obtained by RTA 401 dosed D-9 to D15, which reduced D3+ by 79% ($p < 0.001$), RTA 402 dosed orally at 6.5 mg/kg BID reduced D3+ by 59% ($p < 0.001$). In the fractionated radiation study, RTA 401 dosed from D-9 to D15 reduced D3+ by 52% ($p < 0.001$), and RTA 402 dosed at 6.5 mg/kg PO BID reduced D3+ by 17% ($p = 0.016$). These studies indicate that the radio and chemo-protective effects of RTA 401 and RTA 402 cross multiple clinical settings, and that the agents can be dosed in a variety of schedules before, during, or after radiation. Taken together with other studies of ROS-mediated toxicities, it appears these Phase 1 anti-cancer agents also hold considerable promise in protecting against the side effects of standard therapies, including oral mucositis. Phase 1/2 clinical studies examining both anti-cancer and anti-mucositis endpoints with RTA 401 and RTA 402 are expected to begin in late 2006.

534 POSTER

Targeting CTP synthetase by cyclopentenyl cytosine (CPEC) increases the effectiveness of Gemcitabine and radiation in human tumor cells

C. van Bree[1], H.M. Rodermond[2], R. Leen[3], J.P. Medema[2], A.B.P. van Kuilenburg[3]. [1]*Academic Medical Center-University of Amsterdam, Lab. Exp. Oncology & Radiobiology (LEXOR), Amsterdam, The Netherlands;* [2]*Academic Medical Center-University of Amsterdam, LEXOR, Amsterdam, The Netherlands;* [3]*Academic Medical Center-University of Amsterdam, Genetic Metabolic Diseases, Amsterdam, The Netherlands*

Background: Gemcitabine is a potent enhancer of radiosensitivity but the current clinical efficacy of Gemcitabine and radiotherapy in non small cell lung (NSCLC) and pancreatic cancer is hampered by severe side-effects. To improve its therapeutic ratio, the activation of Gemcitabine can be modulated by pre-incubation with cyclopentenyl cytosine (CPEC). The triphosphate form of CPEC specifically targets cytidine triphosphate (CTP) synthetase leading to decreased cellular CTP-levels. Subsequently, the activity of the rate-limiting enzyme in the activation of Gemcitabine, deoxycytidine kinase (dCK), increases. In this study, we determined the influence of CPEC on the anabolism, cytotoxicity and radioenhancement of Gemcitabine in human tumor cells.
Materials and Methods: Human NSCLC and pancreatic tumor cells were exposed to CPEC, Gemcitabine and radiation. The effects of treatment on CTP-levels, dCK-activity and anabolism of Gemcitabine were assessed by HPLC. Effects on cell cycle distributions were analysed by bivariate flowcytometry. Treatment sensitivity was evaluated by clonogenic and cell death assays.
Results: In NSCLC cells, a 24-hour exposure to CPEC reduced CTP-levels to 10−25% and increased dCK-activity about 2-fold. This resulted in a 4 to 6-fold increase of Gemcitabine incorporated into the DNA ($P = 0.01$). CPEC markedly increased the ability of Gemcitabine to enhance radiosensitivity ($P < 0.001$). CPEC alone enhanced radiosensitivity at doses above 4 Gy ($P = 0.02$). Also in human pancreatic tumor cell lines, CPEC markedly increased the effectiveness of Gemcitabine alone as well as in combination with radiation. Again a 2–3 fold increase in dCK-activity was found, but only after longer exposures to CPEC (48−72 hours). CPEC increased the number of S phase cells, but reduced the incorporation of bromodeoxyuridine. The importance of the scheduling of CPEC, Gemcitabine and radiation together with preliminary data on the effects of CPEC in animal tumor models will be presented.
Conclusions: Targeting the synthesis of CTP by CPEC allows an improved efficacy of Gemcitabine and radiation in different human tumor cells. The demonstration that CPEC increases the therapeutic ratio of Gemcitabine combined with radiation in animal tumor models may provide guidelines for future clinical application.

535 POSTER

AZD2171, a highly potent, orally active VEGF signalling inhibitor, enhances the effect of fractionated radiotherapy in human lung tumour xenografts

K. Williams[1], A. Shannon[1], B. Telfer[1], M. Babur[1], I. Stratford[1], S. Wedge[2]. [1]*University of Manchester, Department of Pharmacy, Manchester, United Kingdom;* [2]*AstraZeneca, Cancer Bioscience, Macclesfield, United Kingdom*

Background: There is an increasing body of evidence suggesting that blockade of the vascular endothelial growth factor (VEGF) signalling cascade enhances the therapeutic effect of radiation. AZD2171 is a highly potent, orally active, VEGF signalling inhibitor that prevents physiological and pathological angiogenesis *in vivo* and significantly inhibits the growth of histologically diverse human tumour xenografts.
Materials and Methods: AZD2171 was analyzed in combination with radiation in Calu-6 (non-small-cell lung cancer) human tumour xenografts. Radiation was administered as 5 consecutive daily fractions of 2 Gy. Chronic, once-daily, oral dosing with AZD2171 (3 mg/kg/day) was initiated 2 hours after completion of the radiotherapy course (sequential regimen) or 2 hours prior to each 2 Gy fraction and continued post-radiotherapy (concomitant regimen). Treatments were initiated at a tumour size of 250 mm^3 (n = 7/group). The experimental endpoint was a quadrupling of tumour volume following the initiation of treatment (RTV4). To assess hypoxia and perfusion of the tumours, pimonidazole and Hoechst 33342 were given to additional animals, treated with concomitant radiotherapy ± AZD2171, prior to sacrifice.
Results: Chronic administration of AZD2171 alone slowed Calu-6 tumour growth significantly (Table 1). When combined with 5×2 Gy there was a positive interaction between the two treatment modalities (Table 1). Both sequential and concomitant regimens resulted in larger average growth delays than would have been predicted from simply combining the growth delays produced by AZD2171 and radiation alone (Table 1). The enhanced response to the combined treatment was associated with an increased tumour doubling time (Table 1) when compared with AZD2171 alone. This suggests that in the Calu-6 model the tumour vasculature may be sensitized to treatment with AZD2171 following fractionated radiotherapy. Histological analyses revealed that concomitant AZD2171 and 5×2 Gy reduced vessel density, with a proportional change in perfusion. This was associated with increases in both tumour hypoxia and apoptosis when compared with radiotherapy alone.

Table 1. AZD2171 combined with 5×2 Gy radiation in Calu-6 tumour xenografts

Treatment	Doubling time (days)	Growth delay, $RTV4_{treated} - RTV4_{vehiclecontrol}$ (days)
Vehicle	6	–
$5\times$ 2 Gy	8	16
AZD2171	12	7
AZD2171 + $5\times$ 2 Gy (sequential)	16	39
AZD2171 + $5\times$ 2 Gy (concomitant)	17	42

Conclusions: These data further support inhibition of VEGF signalling as a means to enhance tumour radiation response *in vivo* and suggest that the antitumour activity of AZD2171 is more pronounced against vasculature treated with extended fractions of radiotherapy.