and a number of HDAC inhibitors are currently in development. Here, we summarize the results obtained with three different HDAC inhibitors (MS-275, SAHA and CI-994) directly compared in four tumor models.
Material and Methods: Four different cell lines (A375, melanoma; A549 and H460, NSCLC; MaTu, breast carcinoma) were grown as xenografts in nude mice. Tumors were treated after establishment, with all agents given daily p.o. Parameters determined were tumor area, tumor weight and body weight. Tumor weights were used for the calculation of a tumor/control ratio (T/C). Oral bioavailability of the compounds was determined by calculating the area under the curve from 0–4h (AUC_{0-4h}). The compounds were given at 50 and/or 100 mg/kg in 30% HP-ß-CD, pH 5.0 to nude mice. Blood samples were taken and analyzed by LC-MS/MS analysis.
Results: The oral bioavailability of MS-275 revealed an AUC of 45.7 μM*h after oral dosing of 50 mg/kg. CI-994 showed a very high bioavailability of 92.4 μM*h and 185.4 μM*h after 50 and 100 mg/kg, respectively. SAHA showed a very low bioavailability with 1.34 and 2.3 μM*h after application of 50 and 100 mg/kg, respectively. In the A375 melanoma model MS-275 revealed a significant dose-dependent efficacy at lower doses (T/Cs 0.18, 0.36, 0.51 for 50, 25 and 10 mg/kg, respectively) whereas SAHA was only effective at higher doses of 50 and 100 mg/kg (T/Cs 0.48 and 0.52). Similarly, in the MaTu breast carcinoma model, MS-275 showed significant efficacy at all three doses used (10, 25 and 50 mg/kg), whereas SAHA showed a statistically significant effect only at higher doses of 50 and 100 mg/kg. In the A549 NSCLC model only MS-275 50 mg/kg showed a statistically significant effect, all other doses of MS-275 and SAHA revealed either no effect or no statistically significant effect. MS-275 was found to generally exhibit greater efficacy than CI-994, although the compounds are structurally very similar. In the A549 model a 2-fold higher dose of CI-994 was needed to achieve the same efficacy as for MS-275 (T/Cs for MS-275 0.24, 0.42, 0.61 for 50, 25 and 10 mg/kg, respectively; T/Cs for CI-994 0.21, 0.37, 0.51 for 100, 50 and 25 mg/kg, respectively).
Conclusion: Although SAHA is described as a highly potent inhibitor of HDACs *in vitro* (IC_{50} 10nM for HDAC1) the head-to-head experiments revealed a lower efficacy *in vivo* than MS-275 in various tumor models after oral application. This observation can be linked to its low bioavailability, as shown for the AUC determination. CI-994 is structurally very similar to MS-275, but was also found to be less effective *in vivo* than MS-275. Currently, we are performing additional experiments to further evaluate the comparative therapeutic potential of these compounds.

60 POSTER

Geldanamycin combined with bortezomib interferes with the ER-associated protein degradation function of valosin-containing protein

E. Mimnaugh[1], W. Xu[1], B. Scroggins[1], X. Yuan[1], T. Prince[2], S. Hartson[2], M. Vos[3], L. Neckers[1]. *[1]National Cancer Institute, NIH, Urologic Oncology Branch, Rockville, MD, USA; [2]Oklahoma State Univ., Dept. Biochem. & Molec. Biol., Stillwater, Oklahoma, USA; [3]National Cancer Institute, NIH, Cancer & Cell Biol. Branch, Rockville, MD, USA*

Inhibition of Hsp90 activity with geldanamycin (GA) and blocking the proteasome pathway with bortezomib (BZ) causes a massive accumulation of misfolded and ubiquitinated cellular proteins. The combination of GA plus BZ also initiates synergistic cytosolic and ER stress responses and promotes the formation of conspicuous, ER-derived cytoplasmic vacuoles. The Hsp90-dependence of the GA-induced vacuolization phenotype was verified by results showing that 17-AAG, radicicol, and analogs of both classes of Hsp90 targeting agents, but not geldampicin, were also capable of inducing cellular vacuolization, provided proteasome activity was partially inhibited by BZ. Additional results now implicate valosine-containing protein (VCP) in the vacuolization phenomenon induced by GA plus BZ. As a crucial participant in ER associated protein degradation (ERAD), VCP, in cooperation with its effector proteins, Ufd1 and Npl4, is responsible for the ATP-dependent retrograde transport of misfolded proteins from the ER prior to their degradation by cytosolic proteasomes. Mutational inactivation of the ATPase domain of VCP, or inhibition of VCP by over-expression of a small VCP-interacting protein (SVIP), promotes cytoplasmic vacuolization of cells that is virtually identical to that caused by GA plus BZ. Cells transiently transfected with flag-tagged SVIP plasmid developed numerous vacuoles that were visualized by anti-flag immunofluorescence, thus localizing VCP to the vacuole membrane. Interestingly, the incidence of vacuolated SVIP-transfected cells was increased several fold by GA. Following exposure to GA plus BZ, but not the individual drugs, a significant quantity of VCP, as well as Hsp90 and Hsp70, was relocalized into the detergent-insoluble pellet fraction of cell lysates, where presumably all three chaperones were associated with aggregated misfolded proteins. Although VCP co-immunoprecipitated with Hsp90 from tumor cell lysates, the overall cellular level of VCP, as well as its association with Hsp90, was GA-insensitive, making it unlikely that VCP is an Hsp90 client protein. We propose that the drug-induced vacuolization is a mechanism cells use to clear misfolded, GA destabilized Hsp90 client proteins from the ER secretory pathway when the ERAD function of VCP is compromised by a backup of proteins resulting from proteasome inhibition. The accumulation of misfolded proteins would eventually become cytotoxic and probably contributes to the demise of tumor cells.

61 POSTER

Mutant-PTEN leads to constitutive integrin-linked kinase (ILK) activity that regulates PKB/Akt activity in glioblastoma cancer cells and targeting ILK results in tumor growth-delay in vivo

L. Edwards[1], B. Thiessen[2], V. Dragowska[1], T. Daynard[3], S. Dedhar[4], M. Bally[1]. *[1]BC Cancer Agency, Advanced Therapeutics, Vancouver, Canada; [2]BC Cancer Agency, Medical Oncology, Vancouver, Canada; [3]QLT, Medicinal Chemistry, Vancouver, Canada; [4]Jack Bell Research Centre, Cancer Genetics, Vancouver, Canada*

Purpose: The tumor suppressor gene PTEN, regulates the phosphatidyl-inositol-3′-kinase (PI3K) signaling pathway and has been shown to correlate with poor prognosis in high-grade astrocytomas when mutational inactivation or loss of the PTEN gene occurs. PTEN mutation leads to constitutive activation of protein kinase B (PKB/Akt) with phosphorylation at the PKB/Akt sites Thr-308 and Ser-473. Integrin-linked kinase (ILK) has been shown to regulate PKB/Akt activity with the loss of PTEN in prostate cancer. Data summarized in this report demonstrates that ILK activity regulates PKB/Akt activity in glioblastoma cells.
Methods: Three human glioblastoma cancer cell lines were used in this study: SF-188, U87MG and U251. U87MG cells were transiently transfected with ILK antisense (ILKAS) using Lipofectamine 2000. Retroviral constructs with either inactive PTEN (U87G129E, U87GR), or empty vector (U87EV) or with an inducible PTEN construct (U87.23) were generously provided by Dr. Michael Wigler. Muristirone A was added to these transfected cells for induction of PTEN expression. Antisense oligonucleotides against ILK (ILKAS) were derived from a patent from ISIS Pharmaceuticals Inc. in which antisense sequence ID no 37 (5′-GAGATTCTGGCCCATCTTCT-3′) was used. ILKAS is a 20mer antisense oligonucleotide (ODN) with a phosphothioate backbone. ILK kinase activity was determined in cell extracts by immunoprecipitation followed by *in vitro* kinase assays. *In vitro* analysis of ILKAS effects included assessments of P-Akt-Ser-473, P-Akt-Thr-308, apoptosis and nuclear morphology. Efficacy experiments were conducted in male SCID/Rag-2M mice bearing U87MG tumors (6 mice per group). Treatments were initiated on day 22-post inoculation. Saline control, ILKAS or antisense controls were administered using a treatment schedule of i.p. injections given once a day for 5 days with two days off, for a 3 week period at a dose of 5 or 10 mg/kg.
Results: The activity of ILK is constitutively elevated in a serum independent manner in PTEN mutant cells, and transfection of wild-type PTEN under the control of an inducible promoter into mutant PTEN cells inhibits ILK activity. Transfection of ILK antisense or exposure to a small molecule ILK inhibitor, suppresses the constitutive phosphorylation of PKB/Akt on Ser-473 in PTEN-mutant glioblastoma cell lines. In addition, the delivery of ILK antisense to PTEN negative glioblastoma cells results in apoptosis. Finally, glioblastomas generated in Rag-2M mice treated with ILK antisense shows tumor growth delay *in vivo*.
Conclusion: Our initial results indicate that therapeutic strategies targeting ILK may be beneficial in the treatment of glioblastomas.

62 POSTER

Isolation of pericytes from vasculature of human lung tumors

R. Bagley, C. Rouleau, S. Morgenbesser, B. Teicher. *Genzyme Corporation, Oncology, Framingham, USA*

Pericytes, also known as mural cells or myofibroblasts, are a key component of human vasculature. Pericytes wrap around the abluminal surface of blood vessels and interact directly with endothelial cells. Pericytes are also associated with tumor vasculature and are an attractive target for anti-angiogenic therapy. We have isolated pericytes from clinical lung samples of patients presenting an adenocarcinoma, a squamous cell carcinoma, or a tumor of neuroendocrine origin. Following surgical excision, tumors were digested with collagenase and elastase. Magnetic beads coupled with cell-specific antibodies were used to deplete blood cells (anti-CD14, -CD45, -CD64), epithelial cells (anti-BerEP4), and endothelial cells (anti-CD31). Remaining cells were placed in culture on poly-L-lysine coated flasks with media that supports pericyte growth and includes FGF, EGF, and IGF-1. Following expansion in culture for 1–2 weeks, pericytes were positively selected using magnetic beads coupled to an antibody against the proteoglycan NG2. These methods resulted in the isolation of a pericyte population with over 90% of the cells expressing NG2. The distinct morphology of the pericytes isolated is consistent with previous reports: elongated cytoplasmic extensions, ruffled membranes, and an