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.

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

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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

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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.

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Isolation of pericytes from vasculature of human lung tumors

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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

often spidery-shape appearance. The expression of markers commonly used to identify pericytes in situ was confirmed by flow cytometry: desmin, alpha smooth muscle actin, VCAM, and fibronectin. Pericytes contribute to vasculature and tube formation is a fundamental process in angiogenesis. The ability of the pericytes isolated from clinical lung samples to form tubes was evaluated in vitro. Pericytes were cultured onto Matrigel, a mixture of basement membrane proteins purified from murine tumors. Pericytes from both normal and diseased tissues formed linear tubes when 20–30,000 cells were seeded into a well of a 48-well plate. In summary, the cells isolated from several human lung tumors possess the characteristics typically associated with pericytes. The ability to propagate pericytes directly from the tumors of cancer patients is a valuable resource that will enhance our understanding of the contributions pericytes make during angiogenesis in malignant phenotypes. Incorporation of such pericytes into drug development programs may lead to more effective cancer therapies that can destabilize tumor vasculature and cause tumor regression.

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Identification of important genes for recurrence of gastric cancer by gene expression profiling

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Background: Recent progress in diagnostic and treatment technologies have enabled us to offer excellent long-term survival results for early gastric cancer, but the prognosis of advanced gastric cancer is still unfavorable. Even after curative resection, 40% of patients with advanced gastric cancer die of recurrence. Cancer is a genetic malady, which progresses through the continuous accumulation of genetic and epigenetic changes. These aberrations may affect the expression of large number of genes. Hence, systematic analysis of gene expression profiling might be beneficial for searching important genes.
Purpose: To search important genes associated with recurrence, we performed gene expression profiling in 60 advanced gastric cancer tissues using a PCR-array system: a high-throughput quantitative RT-PCR technique based on adaptor-tagged competitive PCR (ATAC-PCR).
Materials and Methods: To select only genes actually expressed in gastric tissues, we constructed two cDNA libraries from gastric cancer and normal gastric mucosa. From these two cDNA libraries and literatures described the carcinogenesis or development of gastric cancer, we designed 2304 PCR primers for the ATAC-PCR reaction. We obtained gene expression profiling data from 40 advanced gastric cancer patients (21 recurrence-free cases and 19 recurred cases). To search important genes associated with recurrence, we obtained signal-to-noise ratio and ranked genes. We selected 20 top ranked genes and confirmed the reliability of these selected genes by constructing a molecular-based diagnostic system with these genes. Briefly, by calculating 'prediction strength' ('PS') each case is assigned to 'PS>0' or 'PS<0' groups; in our system, 'PS>0' means a recurrence-free case and 'PS<0' means a recurred case, respectively. We prepared other 20 (11 recurrence-free cases and 9 recurred cases) advanced gastric cancer cases as a validation set and predicted the recurrence. Furthermore, Kaplan-Meier analysis with recurrence was performed.
Results: Selected 20 genes involved the genes reported to be concerned with the development and malignancy of gastric or other cancer, such as ERBB2 and HSP40. These genes revealed distinct expression patterns between recurrence-free and recurred cases. Our diagnostic system correctly predicted recurrence in 15 of 20 cases in the validation set and Kaplan-Meier analysis revealed significant difference between 'PS>0' and 'PS<0' groups.
Conclusions: We selected 20 important genes for recurrence of advanced gastric cancer. These 20 genes might be the potential therapeutic targets for gastric cancer. Our molecular-based diagnostic system is clinically useful to predict recurrence of gastric cancer.

64 POSTER

Assessment of antitumor effects of erlotinib prior to first-line surgical treatment of head and neck squamous cell carcinoma

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Epidermal growth factor receptor (HER1/EGFR) overexpression is implicated in tumourigenesis, including head and neck squamous cell carcinoma (HNSCC). Erlotinib (Tarceva™), a small molecule inhibitor of HER1/EGFR tyrosine kinase (TK), has antitumor activity in several tumour types. However, there is a clinical need to identify markers that predict response, and to further understand pharmacokinetic (PK) and pharmacodynamic properties of this agent class. Patients with HNSCC (diagnosed by pan-endoscopy) requiring cervical lymph node dissection or likely to benefit from curative surgery were studied. Oral erlotinib was administered (150mg/day) for 3–4 weeks prior to surgery. Pre- and post-surgery tumour samples were assessed. The primary endpoint was tumour HER1/EGFR TK inhibition. Secondary objectives included: correlation of PKs with effective biological dose; relationship of biological effect to tumour site and morphology; effects on protein effectors of cell cycle arrest (e.g. P-MAP kinases, cyclins and cyclin-dependent kinases, cycle-progression inhibitors, Ki-67, AKT) and molecular comparison of the HER1/EGFR catalytic domain in normal and tumour tissue. To date, eleven patients have been recruited into this study. A comparative immunohistochemistry (IHC) analysis has been performed on tumour samples collected pre- and post-treatment with erlotinib. In patients responding to erlotinib treatment, dramatic changes in cell proliferation (MAP kinases) and apoptotic pathways (AKT and cell cycle inhibitors p21 and p27) were observed. These changes were not correlated with the initial expression levels of HER1/EGFR. Molecular analysis of HER1/EGFR catalytic domain is currently underway. In conclusion, the available preliminary data suggest that it may be possible to define potential tumour markers to assist in the selection of patients likely to benefit from treatment with erlotinib. Further results from this ongoing trial will be presented.

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Kinomic profiling identifies PKC/Akt and beta-catenin/TCF mediated signal transduction as important targets of celecoxib in colon cancer

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Introduction: NSAIDs and selective cyclooxygenase-2 (Cox-2) inhibitors possess anti-carcinogenic potential against colorectal cancer (CRC). However the molecular targets in cancer of these drugs remain to be elucidated.
Aims & Methods: We hypothesized that celecoxib, a selective cox-2 inhibitor directly targets the activity of kinase proteins resulting in apoptosis and downregulation of Wnt signaling. A new kinase substrate peptide array consisting of 1200 peptides with specific phosphorylation sites was used to comprehensively evaluate the effects celecoxib on the entire kinome in the colon cancer cell lines HT29 and DLD-1. Results of the kinome array were validated with Western blot analysis. To study β-catenin/TCF dependent transcription, a luciferase reporter assay was used. We evaluated the expression of oncogenes with quantitative PCR. Apoptosis was measured by the level of cleaved caspase 3.
Results: Celecoxib has important anticancer mechanism since apoptosis and cell cycle arrest was induced at low levels of celecoxib, 25 μM. The kinase array analysis revealed inhibition of the kinases IGF-r, Act, PKC and upregulation of GSK3 in the presence of celecoxib, independent of Cox-2 expression. Moreover an increase phosphorylated β-catenin was observed within 60 minutes. This effect of celecoxib was accompanied by a downregulation of the β-catenin/TCF dependent transcription. Subsequently expression levels of the oncogenes cyclin d-1, c-Myc and c-Met were reduced.
Conclusion: Celecoxib directly inhibits the activity of the IGF-r, PDK1/2, Akt and PKC. In addition GSK3 activity was enhanced which can explain the increase of phosphorylated β-catenin. Celecoxib caused a downregulation of beta-catenin–TCF dependent transcription of oncogenes at relatively low levels independent of tumour-Cox-2. Hence we have identified a possible link between Akt and Wnt signal transduction which can explain the chemopreventive and anticarcinogenic properties of celecoxib in colon cancer. This study provides a novel mechanism of action of celecoxib in colon cancer.

66 POSTER

Anti-proliferative activity of a PPAR gamma agonist is associated with changes in the expression of cell cycle and apoptosis related genes in human ovarian cancer cells

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Ovarian cancer is the most common cause of cancer-related death in women. Current therapies for advanced ovarian cancer are clearly inadequate and new molecular-targeted agents need to be evaluated for treatment of this disease. The peroxisome proliferator-activated