349 POSTER

Vorinostat sensitizes colorectal cancer cell (CRC) lines to AZD6244 and results in synergistic inhibitory effects on proliferation

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Background: AZD6244 (ARRY-142886) is a potent and selective small molecule inhibitor of MEK1/2 with nM potency against CRC cancer cell lines in vitro and is active in several in vivo models of CRC. In phase I trials of AZD6244, inhibition of p-ERK was noted in PBMCs and tumor biopsies at tolerable dose levels, with the best outcome being stable disease. Despite the initial enthusiasm for this class of agents in RAS or BRAF mutant tumors, it is clear that new approaches, including combination therapies, are needed. Vorinostat (Vo) is an inhibitor of histone deacetylases (HDACs) and has been shown to cause growth arrest of cell lines derived from several human cancers including, colon, pancreatic and hepatocellular carcinoma. The goal of this study was to assess the effectiveness of combining AZD6244 with Vo in CRC cell lines.
Methods and Results: Initially, we assessed sensitivity to varying doses of AZD6244 or Vo as single agents against a panel of CRC cell lines. Inhibition of proliferation was determined and cell lines were designated sensitive (S, IC50 <1.0 μM) or resistant (R, IC50 >1 μM) for each drug. We then selected cell lines according to the following conditions: (1) S to both drugs, (2) R to both drugs, (3) S to AZD6244 but R to Vo, and (4) R to AZD6244 but S to Vo, and treated them with varying doses of the combination of the two drugs. The combination of AZD6244 and Vo resulted in synergistic inhibition of proliferation in cell lines that were previously R to AZD6244 but S or R to Vo, as determined by the method of Chou and Talalay. Cell lines that were S to single agent AZD6244 also demonstrated a synergistic effect when combined with Vo with combination indices <1.0. Strikingly, the SW480 cell line, which was R for both drugs as single agents, was converted to S in response to the combination treatment. To determine the effects on known target proteins of these drugs we performed western blot analysis for phospho-ERK (pERK) and actetylated histone H3 in SW480 cells treated with varying doses of AZD6244 or Vo as single agents and in combination. Single agent AZD6244 treatment resulted in an expected dose-dependent decrease in pERK, and histone H3 acetylation was similarly increased with single agent Vo. Interestingly, we also observed a significant increase in pERK levels in cells treated with Vo alone, which may indicate an increased dependence on the MEK pathway as a result of the inhibition of alternative pathways through modulation of HDACs.
Conclusions: We determined the combination of AZD6244 with Vo resulted in synergistic inhibition of proliferation of CRC cell lines. Lines that were R to either drug alone were converted to the S phenotype when treated in combination. Vo increased pERK levels, which we hypothesize may be involved in sensitizing the cells to AZD6244. Our pre-clinical results suggest that combination treatment with AZD6244 and Vo may be a more effective therapy for treatment of CRC versus either agent alone.

350 POSTER

Measurement of mechanistic markers of histone deacetylase (HDAC) inhibition in samples from clinical trials

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Introduction: Assays used to analyse clinical samples should be robust, sensitive and quantitative. Western blots are the 'gold standard' but are slow, have low throughput and are not easily quantifiable. Other assay technologies e.g. ELISAs offer improved characteristics for analysis of clinical samples. HDACs are chromatin modifying enzymes which modulate the transcriptional activity of genes important for tumour development, growth and survival. Increased acetylation of histones is an important marker of HDAC inhibition.
Methods: We have optimised a quantitative Meso Scale Discovery sandwich ELISA for acetylated histone H3 (AcH3), the results of which are quantitated by the inclusion of a calibration curve. Immunofluorescence (IF) has been used to measure AcH3 levels in plucked eyebrow hair follicles. AcH3 was detected using primary antibody (Upstate 06–599) and secondary antirabbit IgG conjugated to Alexa 488 (Invitrogen). TO-PRO-3 (Invitrogen) was used as a nuclear marker. AcH3 was visualised using confocal microscopy (Leica SP2). Fluorescence intensity in hair follicles was quantified using INCell Translator (GE Healthcare).
Results: Using ELISA, AcH3 increased 3 and 1.5-fold following exposure of HCT116 human colorectal tumour cells to the HDAC inhibitors SAHA and MS-275 (5XGI50 for 24 h) respectively. This assay was suitable for measuring changes in AcH3 in peripheral mononuclear cells (PMC) treated ex vivo with the same HDAC inhibitors for 4 h and was validated for Good Clinical Laboratory Practice (GCLP). Within and between plate variation was 9.6%, and 12.5% respectively with a recovery for a 60 ng/ml AcH3 peptide spike of 113±32.7%. The assay was used to measure changes in AcH3 in PMC samples from patients taking part in a Phase 1 clinical trial of an HDAC inhibitor (R306465). A minimal rise in AcH3 was detected in patients receiving the lowest drug doses (100, 200 and 300 mg). A 5−10 fold increase in AcH3 was measured in 2/6 patients receiving the highest dose (400 mg). The assay has been re-optimised for increased sensitivity and will be used to measure samples from future HDAC inhibitor clinical trials. Hair follicles offer an alternative surrogate tissue for measurement of mechanistic markers. Nuclear AcH3 increased 5-fold in plucked hair follicles treated ex vivo with SAHA (5xGI50 for 4 h).
Conclusion: This IF technique will be used in future clinical trials of HDAC inhibitors and the results compared to those obtained with the ELISA.

351 POSTER

Identification of kinases that are potential molecular determinants of cellular response to radiation using antibody arrays

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Background: Deregulated expression and activity of receptor tyrosine kinases (RTKs) and associated proteins are implicated in carcinogenesis, cancer progression and metastasis of several human malignancies, including head and neck squamous cell carcinomas (HNSCCs). Resistance of HNSCCs to therapy is a major challenge and recent reports show that redundant and overlapping signal transduction pathways shared by various kinases are responsible for poor treatment outcome. The goal of this study is to identify kinases that may play a role in cellular response to radiation therapy. This will enable us to develop novel multimodality treatment strategies to overcome cancer cell resistance to therapy.
Materials and Methods: Four HNSCC lines (HN-5, Fadu, HN-30 and UMSCC-10) were used for the study. Clonogenic cell survival assays were performed to characterize these HNSCC lines for their response to radiation (2−6 Gy). Lysates of untreated or irradiated cells (collected 2 or 30 min after 4 Gy) were analyzed by antibody arrays (Proteome ProfilerTM, R&D Systems, Inc., Minneapolis, MN, USA) generated for quantifying expression levels of phosphorylated RTKs (human phospho-RTK array kit) and phosphorylated forms of other kinases (human phospho-kinase array kit). Western blot analysis was performed to validate the expression levels.
Results: Clonogenic cell survival data showed that HN-5 and Fadu cells were relatively radioresistant with surviving fractions after 2 Gy (SF2) of 0.82 and 0.65 respectively. HN-30 and UMSCC-10, were more sensitive with SF2 of 0.5 and 0.52 respectively. Among the RTKs investigated, basal levels of EGFR, ErbB3, VEGFR3, EphA7 and Dtk were significantly over-expressed in HN-5 and Fadu cells than in HN-30 and UMSCC-10 cells. Among other associated kinases, cSrc was over-expressed in HN-5 and Fadu cells and Erk1/2 was over-expressed in HN-30 and UMSCC-10 cells. Exposure to radiation resulted in up-regulated phosphorylation of EGFR, ErbB3, VEGFR3, cSrc, and Chk-2. Among these proteins, EGFR, ErbB3, VEGFR3 and cSrc were validated by western blot analysis.
Conclusions: Our data showed that (1) antibody arrays serve as a powerful tool to determine the expression levels of phospho kinases (2) our data suggest that EGFR, ErbB3, VEGFR3, and cSrc may contribute to governing cellular responses to radiation. Further investigation on specific role for these proteins in cell radiosensitivity is underway.
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352 POSTER

Small molecule antagonists of very late antigen (VLA)-4 inhibit metastasis formation and tumor growth of melanoma

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Very late antigen-4 (VLA-4) constitutes an interesting molecular target for innovative therapy against melanoma progression. In previous studies, we have reported that treatment of melanoma cells with a novel family of VLA-4 antagonists abrogate their adhesion to cytokine-activated endothelial cells and efficiently inhibit their metastatic development in vivo. Other studies evidenced that VLA-4 is an endothelial cell receptor that modulates angiogenesis. Herein, we have evaluated the in vivo antimetastatic and