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Downstream mechanistic markers of HDAC inhibition for drug discovery and clinical evaluation

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Post-translational modifications on histone proteins function as a 'histone code' to alter chromatin architecture and gene transcription. As the underlying cause of a large number of cancers is aberrant gene expression, one therapeutic tactic is to manipulate the histone code by developing small molecule inhibitors against chromatin modifying enzymes. Success in this field is exemplified by histone deacetylase inhibitors (HDACIs), e.g. the hydroxamate SAHA and the benzamide MS-275 which are undergoing clinical evaluation. Chromatin modifying enzymes also target non-histone proteins, including chaperone (e.g. HSP90), structural (e.g. α-tubulin) and transcription factor (e.g. p53, HIF-1α) proteins, A key requirement of mechanism-based drug discovery is to demonstrate that investigational compounds affect drug targets in the expected manner. We have evaluated ELISA methodologies (time-resolved fluorescence cell-based ELISAs (TRF-Cellisas and DELFIA assays) and the multiplexed assay platform, MesoScale Discovery (MSD), as tools for measuring biomarkers of HDAC inhibition. Histone H3 acetylation increased following exposure ($5\times$GI50 for 24h) of human tumour cells (HCT116) to SAHA (3-fold) and MS-275 respectively. These changes were similar to those measured by western blotting. A quantitative MSD assay was also suitable for measuring changes in H3 acetylation in peripheral mononuclear cells treated *ex vivo* with the same HDACIs for 4h. One key question for HDACI development is the need to define isoform selectivity. We have adapted the TRF-Cellisa to measure histone and α-tubulin acetylation, potential biomarkers of pan-HDAC and HDAC6 inhibition respectively. SAHA increased H3 and α-tubulin acetylation to similar extents whereas the non-HDAC6 inhibitor, sodium butyrate, only increased H3 acetylation. Eighteen hydroxamic acids (20 μM), identified from a focussed HDAC biochemical screen, increased acetylation of H3 (1.5−2.1-fold) and α-tubulin (1.6−2.5-fold) compared to control after 4h and 24h exposure. H3 and α-tubulin acetylation could also be quantified using the MSD in human prostate tumour cells (PC3LN3) grown as xenografts and treated with SAHA (50–100 mg/kg $\times$1 for 9 days). The MSD assay platform provides a sensitive, quantitative alternative to western blotting for measuring the downstream effects of HDACIs. We are presently validating assays for Good Clinical Laboratory Practice for use in future clinical trials.

Supported by CRUK C309/A2187.

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Modulation of estrogen and progesterone receptor signaling by selective HDAC inhibition

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Background: Modulation of estrogen signaling is one of the most successful modalities in the treatment of estrogen-receptor (ER) positive breast cancers. However, many ER-positive tumors show primary or acquired resistance to the commonly used anti-estrogens (e.g. tamoxifen, TAM) or aromatase inhibitors (AI). Particularly, tumors that express ER but not progesterone receptor (PR) may be more resistant to therapy.

Histone deacetylases (HDAC) play a central role in transcriptional regulation and may be involved in the pathogenesis of breast cancer. Several investigators have shown that HDAC inhibitors (HDACi) may restore sensitivity to anti-hormonal therapy. However, the mechanism and role of PR in this interaction and the relevance of individual HDAC enzymes remain unclear.

Here, we evaluated the roles of individual HDAC enzymes and the consequence of their inhibition on ER- and PR-mediated signaling in hormone-sensitive and hormone-resistant breast cancer cell lines. These findings are further evaluated in an ongoing clinical trial evaluating the HDACi, Vorinostat (SAHA) and tamoxifen after AI failure.

Methods: The roles of individual HDAC enzymes on ER and PR expression in pre-specified breast cancer cell lines were evaluated by selective inhibition of HDACs using siRNA and the use of various classes of HDACi.

Results: The co-treatment of both ER-positive and ER-negative breast cancer cell lines with HDACi and TAM resulted in synergistic anti-tumor activity. Treatment of ER-positive cells with HDACi resulted in down-regulation of ER with minimal effects on PR. In contrast, HDACi treatment of ER-negative cells resulted in a down-regulation of PR. Selective depletion of HDAC1 resulted in down-regulation of ER, but did not affect PR expression, whereas the selective depletion of HDAC2 resulted in reduced expression of both, ER and PR. HDAC6 depletion had no effect on ER. Furthermore, the selective inhibition of HDAC1 and HDAC2, but not HDAC6 by siRNA depletion was associated with growth inhibition even in the absence of TAM.

Conclusions: The anti-tumor effects of TAM in breast cancer cell lines are enhanced by co-administration of HDACi irrespective of ER expression, and are mediated through selective inhibition of specific HDAC enzymes. The synergistic effects observed in ER-negative tumors are mediated through modulation of PR. These findings are currently being evaluated in a clinical trial.

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YM155, a novel small molecule survivin suppressant, exhibits curative antitumor activities in experimental human malignant lymphoma models in vivo

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Survivin is a member of the Inhibitor of Apoptosis (IAP) family of proteins, and is highly expressed in all primary tumor types. Given its preferential expression in tumor cells, and its ability to block apoptosis and regulate cancer cell proliferation, survivin appears to be a putative novel target for cancer therapy. YM155 is a novel, small molecule survivin suppressant (AACR-NCI-EORTC 2005, Abstract #B203). In a phase I, open-label study of YM155 with 7-day continuous i.v. infusion, partial response was observed in the patient with diffuse large B cell lymphoma (DLBCL) refractory to R-CHOP (ASCO Annual Meeting 2006, Abstract #3014). In this study, we evaluated pre-clinical efficacies of YM155 on experimental human DLBCL (RL) and Burkitt's lymphoma (Ramos) models. These two human lymphoma cell lines were either s.c. or i.v. inoculated to mice. Dose level and schedule of each drug was adjusted to clinical equivalent dose. Single administration of YM155 (7-day continuous s.c. infusion) at 3 mg/kg, vincristin (i.v.) at 0.5 mg/kg, or rituximab (i.p.) at 50 mg/kg was repeated for every three-week schedule in mice. We also conducted quantitative real time PCR and immunohistochemical analyses on survivin in the tumors obtained from the YM155-treated mice. In nude mice bearing RL and Ramos lymphoma xenografts, YM155 at 1 and 3 mg/kg induced statistically significant tumor regression with no decrease in body weight at day 21. YM155 suppressed survivin expression, and induced cell death in the tumors obtained from the YM155-treated mice. In addition, some of the YM155-treated animals cured and experienced complete regressions of tumors (4 of 6 animals in RL, 5 of 6 animals in Ramos). In SCID mice i.v. inoculated RL and Ramos, YM155 showed a significant survival benefit compared with control. Vincristine or Rituximab showed no superior antitumor activity or survival benefit to YM155 in all the evaluation models tested. These results clearly support the phase I clinical efficacies of YM155 observed in the patients with DLBCL. In conclusion, YM155 is expected to become a new chemotherapeutic option which can eradicate R-CHOP refractory and aggressive lymphomas in clinical setting.

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Sequence dependent anti-tumor activity of bortezomib and the KSP inhibitor, SB743921, in a solid tumor xenograft model

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SB-743921 is a potent specific kinesin spindle protein (KSP) inhibitor currently in Phase I/II clinical trials. KSP is a kinesin required for completion of mitosis, and KSP inhibitors cause mitotic arrest of tumor cells. Pre-clinical studies suggest that inhibiting exit from a KSP inhibitor induced mitotic arrest increases cancer cell toxicity. We therefore tested combinations of KSP and proteasome inhibitors in tissue culture. Exposure of cells to a KSP inhibitor followed by a proteasome inhibitor 24 hours later resulted in increased cancer cell death compared to the reverse order of addition.

Based upon sequence dependent activity *in vitro*, we initiated combination studies in nude mice, administering both drugs on q4dx3 schedule simultaneously and separated by 24 hours in both orders of addition. Studies to establish the maximum tolerated dose (MTD) of simultaneous and sequenced administration of SB-743921 and bortezomib identified a marked sequence dependence. Administration of bortezomib prior to SB-743921 was least well-tolerated, while on the reverse sequence both drugs could be administered at their respective single agent MTDs.

Anti-tumor activity of sequenced and simultaneous administration was explored in HT29 tumor xenografts. There were two objectives of this study, 1) to compare single agent activity to the combination of SB-743921 administered prior to bortezomib at their respective MTDs, and