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Comparison of the cellular and biochemical properties of ansamycin and non-ansamycin based Hsp90 inhibitors

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Background: Heat shock protein 90 (Hsp90) has emerged as an important target for the treatment of cancer due to its essential role in several key oncogenic signaling pathways. Several classes of Hsp90 inhibitors have recently advanced into clinical trials including ansamycin derivatives that are semi-synthetic derivatives of the natural product geldanamycin (e.g. 17-AAG, IPI-504, 17-DMAG) or synthetic small molecules (e.g. purine derivatives, isoxazoles, pyrazoles). Ansamycin derivatives are potent Hsp90 inhibitors that demonstrate selective cell growth inhibition toward cancer cells as compared to normal cells. We have determined the biochemical and cellular properties of a group of published Hsp90 inhibitors, including both natural product derived and synthetic compounds.
Materials and Methods: The biochemical affinity of inhibitors to Hsp90 was determined using a competition binding assay using radioactively labeled 17-AAG and Hsp90 purified from Hela cells. The growth inhibition induced by Hsp90 inhibitors were evaluated in human normal and cancer cells and the binding of Hsp90 inhibitors to the intracellular pool of Hsp90 in living cells was determined using radiolabeled Hsp90 inhibitors.
Results: The biochemical affinities to purified Hsp90 for the inhibitors tested range from 0.1 to 500 nM. There is a rough correlation between biochemical affinity and cell growth inhibition of cancer cells with a relative activity ranking of isoxazoles > ansamycins > purines. While ansamycins demonstrate selective growth inhibition of cancer cells compared to normal cells as previously described in the literature, the more potent isoxazole derivative also potently inhibits the growth of some normal cell types. To investigate the mechanism of cancer cell selectivity of ansamycin derivatives, the binding of 17-AAG to the intracellular pool of Hsp90 in cancer and in normal cells was measured. While both cell types contain large amounts of Hsp90, 17-AAG binds potently only to the intracellular Hsp90 pool in cancer cells but not in normal cells. We are currently investigating the hypothesis that the increased binding affinity of the isoxazole compound for purified Hsp90 leads to increased Hsp90 binding of this compound even in normal cells.
Conclusion: The experiments presented above raise the question of whether synthetic Hsp90 inhibitors with high affinity for Hsp90 have lost some of the in vitro therapeutic window between cancer and normal cells that makes Hsp90 inhibitors such attractive candidates for cancer therapeutics.

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Acetylation of molecular chaperones by histone deactylase inhibitors (HDACI)

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Background: HDACI have shown anti-tumour activity in patients with hematologic malignancies and solid tumours. SAHA is an FDA approved HDACI but the mechanisms underlying its activity are imprecisely defined. Hsp90 is a molecular chaperone essential for the folding, stability and function of a range of proteins including ERBB2, CRAF and CDK4. Hsp90 client proteins are involved in signalling pathways commonly deregulated in cancer and Hsp90 inhibitors have emerged as attractive anti-cancer agents. It is reported that HDACI cause increased acetylation of Hsp90, decreased ATP binding and a molecular signature consistent with Hsp90 inhibition i.e. depletion of client proteins and induction of Hsp70. HDAC6 has been identified as an Hsp90 deacetylase but the histone acetyltransferase (HAT) responsible for its acetylation has not yet been identified.
Methods: To identify candidate HAT(s) responsible for Hsp90 acetylation in HDACI treated cells, recombinant Hsp90 was incubated with [3H]-acetyl coenzyme A (0.5microCi) and recombinant HAT enzymes p300 and PCAF and the incorporation of radiolabel determined in a filter assay. Immunoprecipitation, western blotting and RTPCR have been used to follow the effect of HDACI on the expression of Hsp90-related proteins in HCT116 human tumour cells.
Results: Hsp90 was acetylated in a concentration-dependent manner by both PCAF and p300. Time-dependent increased Hsp90 acetylation was blocked by peptide Coenzyme A-based HAT inhibitors. Targeted inhibition of p300 in HCT116 cells using siRNA confirmed its role in Hsp90 acetylation. This result was confirmed in an isogenic pair of HCT116 cells one of which had lost p300 by homologous recombination. Interestingly, western blot analysis showed that reduced Hsp90 acetylation in p300 null cells following exposure to SAHA had no effect on the pattern of client protein depletion. Similarly, HCT116 cells exposed to MS275 (5xGI50), a class I inhibitor that does not inhibit HDAC6, showed decreased acetylation of Hsp90 but client protein depletion was still observed. HDACI-induced acetylation of the Hsp90 cochaperones, p23 and HSP72, appeared to be more robust than Hsp90 acetylation.
Conclusions: Acetylation of Hsp90 is mediated at least in part by p300. HDACI results in a molecular signature that is similar to but not identical with that of an Hsp90 inhibitor. The role of cochaperone acetylation remains to be determined.

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MPC-3100: A non-natural product Hsp90 inhibitor with anti-tumor activity in pre-clinical models

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Background: The molecular chaperone Hsp90 stabilizes many proteins subserving tumor cell proliferation and survival, and we have synthesized purine-based inhibitors that show good activity in competitive binding, cellular Her2-Luciferase reporter, and cytotoxicity assays.
Materials and Methods: The Her2-Luciferase reporter consists of the kinase domain of the Hsp90 client protein Her2 fused to Luciferase. Cells or mice were treated as indicated, and cells or tissues processed for Luciferase activity. Hsp70 mRNA was extracted from mouse liver and measured by quantitative RT-PCR. Female nu/nu athymic mice were used as hosts for subcutaneously implanted tumor cells. At a median tumor volume of approximately 100 mm3, dosing was initiated using a 200 mg/kg p.o. qd, 5-days-on/2-days-off schedule for three cycles.
Results: Her2-Luciferase inhibition precedes loss of cell viability, consistent with target-mediated cell killing. Mouse pharmacokinetic studies show that compounds in this class are retained in tumor xenografts at high levels 48 h post dosing, while plasma levels are almost undetectable, and rat pharmacokinetic studies indicate good oral bioavailability of selected compounds (data not shown). As a means to predict in vivo activity of compounds, mice were given a single dose of 100 mg/kg p.o. and then sacrificed 6 h later, at which time quantitative RT-PCR was used to determine the level of Hsp70 induction, a marker of Hsp90 inhibition. MPC-3100 showed a superior pharmacokinetic and pharmacodynamic profile and was taken into pre-clinical anti-tumor models. In HT-29 tumor-bearing mice, growth inhibition relative to vehicle-treated animals was significant at the end of dosing (68%, $p < 0.01$), as well as nine days later (65%, $p < 0.05$). After this point, xenograft growth resumed at a rate similar to vehicle-treated animals. Animals showed no weight loss and tolerated the dosing regimen well. MPC-3100 was also tested in a NCI-N87 Her2+ gastric colon carcinoma xenograft model, and at the end of dosing, the MPC-3100-treated cohort showed tumor shrinkage (44% regression, $p < 0.0001$) with no significant weight loss. In comparison, tumors in 5-FU-treated mice (100 mg/kg i.p. weekly for three doses) did not shrink but grew slowly (91% tumor growth inhibition), and these mice experienced significant weight loss.
Conclusions: The orally bioavailable Hsp90 inhibitor MPC-3100 demonstrates anti-tumor activity using dosing regimens that are well tolerated.

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Efficacy of panobinostat (LBH589) in lung cancer: potent anticancer activity in both in vitro and in vivo tumor models

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Background: Panobinostat (LBH589) is a highly potent deacetylase inhibitor that induces cell-cycle arrest, differentiation, and apoptosis of cancer cells and has demonstrated preliminary clinical efficacy in patients with cancer. Panobinostat affects multiple oncogenic pathways, including induction of cell-cycle arrest, cell apoptosis, and inhibition of angiogenesis. Panobinostat has been shown to sensitize human non-small-cell lung cancer (NSCLC) cell lines to radiation-induced DNA double-strand breaks and to synergize with erlotinib to cause apoptosis of EGFR-dependent lung cancer cells. In this study, we further investigated the effects of