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Chemosensitization by targeting a protein phosphatase in human cancer cells

Y. Liao[1], Y. Wu[2], B. Ping[3], Y. Wei[1], L.A. Donehower[4], M.C. Hung[1]. *[1]The University of Texas M.D. Anderson Cancer Center, Molecular & Cellular Oncology, Houston, TX, USA; [2]The University of Texas M.D. Anderson Cancer Center, Pathology, Houston, TX, USA; [3]Cancer Hospital, Fudan University, Pathology, Shanghai, P.R. China; [4]Baylor College of Medicine, Molecular Virology & Microbiology, Houston, TX, USA*

Background: Overexpression of a wild-type p53-inducible serine-threonine phosphotase Wip1, through gene amplification has recently been found in a variety of human cancers, including neuroblastomas (28%), breast (16%) and ovarian (25%) cancers. The Wip1 gene is located in the 17q23 amplicon, a novel region of amplification that has been linked with poor prognosis of breast and ovarian cancer patients. Although expression of Wip1 is induced by p53 following irradiation, it negatively regulates p53 functional activation. In addition, Wip1 also negatively regulates cell cycle checkpoints via dephosphorylation of p53 protein, p38, Chk1, and Chk2 kinases. It is very likely that overexpression of Wip1 in human tumors may confer resistance to DNA damaging drugs (such as cisplatin, adriamycin, VP-16, etc) and microtubular inhibitors, such as paclitaxel. Therefore, targeting Wip1 should be a mechanism-based, rational approach to improve the efficacy of anti-cancer therapeutics.
Materials and Methods: Human cancer ell lines and mouse embryonic fibroblasts (MEF) cells with Wip1 (−/+) and Wip1 (−/−) were used. Expression levels of Wip1, p38, and p53 were detected by Western blot. Flow cytometry and antibodies against cleaved PARP were used to quantify cell death by apoptosis before or after exposure to drugs in the presence or absence of Wip1 siRNA.
Results: By staining tissue microarray slides, we demonstrated a close correlation between the expression levels of Wip1 with tumor stage in human cancer. When exposed to anti-cancer drugs, the Wip1 (−/−) MEF cells were more sensitive to anticancer drug-induced apoptosis than the Wip1 (−/+) MEF cells. While re-introducing Wip1 expressing by transfecting a pcDNA3-Wip1 expression plasmid construct into the Wip1 (−/−) MEFs confers resistance to drugs. Similarly, when knocking down Wip1 expression in Wip1 overexpression cancer cells, such MCF-7, we observed a synergistic effect of siRNA against Wip1 combined with anticancer drugs. Expression of p53 protein and phospho-p38 were elevated when Wip1 was knocking down by siRNA. In addition, the sensitivity and percentage of apoptotic cells correlated with the relative expression levels of phospho-p38, p53, and Wip1 protein in the presence or absence of anti-cancer drugs.
Conclusions: Our data suggest that targeting Wip1 expression is a novel approach for sensitization of Wip1 overexpressing cancer cells to anti-cancer drugs.

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Oral in vivo pharmacokinetics of the novel orally available taxane derivative BMS-275183 in wildtype and MDR1a/b double knockout mice support that BMS-275183 is a P-glycoprotein substrate drug

S. Marchetti[1], D. Pluim[1], O. Van Tellingen[2], L. Alland[3], J. Gan[3], S. Galbraith[3], J.H. Beijnen[4], R. Mazzanti[5], J.H.M. Schellens[4]. *[1]The Netherlands Cancer Institute, Experimental Therapeutics, Amsterdam, The Netherlands; [2]The Netherlands Cancer Institute, Clinical Chemistry, Amsterdam, The Netherlands; [3]Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ, USA; [4]The Netherlands Cancer Institute, Medical Oncology, Amsterdam, The Netherlands; [5]University of Florence, Section of Medical Oncology, Postgraduate School of Oncology, Florence, Italy*

Introduction: Currently, the possible implications of affinity of BMS-275183 for P-glycoprotein (Pgp) in the oral bioavailability and drug-drug interactions between BMS-275183 and benzimidazole proton pump inhibitors (PPIs) are being investigated, as in ongoing clinical trials co-administration of a PPI is associated with elevated exposure to BMS-275183 and toxicity.
Methods: *In vitro*: First, we investigated whether the interaction takes place at the level of Pgp (MDR1), BCRP (ABCG2) and MRP2, which are expressed apically in the gut epithelial layer. BMS-275183 was used in *in vitro* cell survival experiments with wildtype and –MDR1, or –BCRP, or –MRP2 overexpressing MDCKII cells. Active drug transport in monolayers of wildtype and MDR1 overexpressing LLCPK cells (LLCPK-MDR1) and in wildtype and MDR1, or BCRP, or MRP2 overexpressing MDCKII cells in monolayers was also tested.
In vivo: Then, we determined the oral pharmacokinetics (PK) of BMS-275183 (10 mg/kg) *in vivo* in wildtype (wt) mice and in the mdr1 double knockout mice (Mdr1a/1b-/-) in the presence and in the absence of the orally administered PPI pantoprazole (40 mg/kg).
Results: *In vitro:* IC50 values obtained with the SRB assay of BMS-275183 in MDCKII-wildtype vs –MDR1 were 2.0±0.5 and 28±3.4 nM, respectively (resistance factor RF=14; $p < 0.01$). Results with the control substrate paclitaxel revealed an IC50 of 15±10 in MDCKII-wildtype and 585±88 nM in MDCKII-MDR1 (RF = 39; $p < 0.01$). IC50 values in MDCKII-BCRP vs. MDCKII-wildtype cells were not different ($p < 0.2$). BMS-275183 was actively transported by MDR1 in LLCPK-MDR1 and MDCKII-MDR1, at a level that was slightly lower than that of paclitaxel. Active transport was completely inhibited by pantoprazole (1.25 mM; MDR1 and BCRP inhibitor), or zosuquidar (5 μM; MDR1 inhibitor). Moderate transport was also found in MDCKII-MRP2, which could completely be inhibited by the MRP2 inhibitor MK571 (50 μM).
In vivo, after an oral dose of 10 mg/kg, the AUC values of BMS-275183 based on a minimum of 3 mice per time point were 4665±789 (mean±SE; ng h/ml) in wt and 8761±406 (ng h/ml) in mdra/b KO mice ($p < 0.01$). Co-administration of one dose of 40 mg/kg of oral pantoprazole did not affect the AUC values in wt mice. CNS concentrations of BMS in wt mice were below the lower limit of quantification of the assay, but were 851±442 and 932±429 ng/g tissue at 1 and 4 h resp. in the KO mice. Pantoprazole had no significant effect on CNS concentrations in wt and KO mice.
Conclusions: It is demonstrated that BMS-275183 is a good substrate for MDR1 in both *in vitro* and *in vivo* experiments. *In vitro*, it is also a moderate substrate for MRP2 and not a substrate for BCRP. Although co-administration of pantoprazole did not have any effect on the oral exposure and brain penetration of BMS-275183 it is warranted to further explore the *in vivo* interaction between BMS-275183 and PPIs in patients, because of the possible interaction suggested by prior clinical experience.

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EGFR activation and ERK survival signaling is triggered by proteasome inhibitors: implications for combining proteasome inhibitors with EGFR inhibitors

J. Cusack[1,2], F. Wang[1,2], C. Sloss[1,2], R. Lu[1], L. Xia[1]. *[1]Massachusetts General Hospital, Department of Surgery, Boston, USA; [2]Harvard Medical School, Boston, USA*

Background: Inducible chemotherapy resistance mediated by the activation of the transcription factor NF-B in response to genotoxic chemotherapy may account for a substantial number of treatment failures in pancreatic cancer and other malignancies. Inhibition of NF-B has been shown in preclinical models to overcome this inducible treatment resistance and promote the apoptotic response to chemotherapy treatment. Previous studies suggest that proteasome inhibitors augment chemosensitivity in part by inhibiting chemotherapy induced NF-B activation. Proteasome inhibitors are also known to promote apoptosis by suppression of (EGFR) endocytosis and degradation. The current study was performed to evaluate the effect of proteasome inhibitors on EGFR and ERK survival signaling in pancreatic cancer cells.
Materials and Methods: The effects of proteasome inhibitors on EGFR and ERK signal transduction were determined by western blot analysis of total and phospho-EGFR and phosphor-ERK in the presence and absence of EGFR tyrosine kinase inhibitors. The effects of treatment on apoptosis were determined using the cell-death ELISA assay. The effects of combination treatment were also assayed using Panc-1 xenografts grown in nude mice.
Results: Proteasome inhibition using salinosporamide A (NPI-0052) or bortezomib (velcade®) up-regulated the phosphorylation of EGFR. The downstream survival signaling pathway of ERK was also activated. When proteasome inhibition was combined with an EGFR tyrosine kinase inhibitor using either erlotinib (Tarceva®) or gefitinib (Iressa®), apoptosis and tumoricidal response were significantly enhanced.
Conclusions: EGFR tyrosine kinase inhibitors augment the apoptotic response to proteasome inhibition by blocking survival signals that result from treatment-induced activation of EGFR and ERK signal transduction in pancreatic cancer models. The inclusion of an EGFR TKI to proteasome inhibitor-based treatment regimens should be considered.