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Selective PIN1 PPIase inhibitor, AG122005, causes a p53 independent p21/waf1/cip1 induction

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PIN1, a resident nuclear protein, has a role in the regulation of the cell cycle and may have oncogenic potential being over expressed in a wide variety of transformed cell types. PIN1 has recently been identified as a critical regulator of the tumor suppressor p53 during DNA damage response. Following its stress-induced phosphorylation by p38 MAPK, p53 forms a complex with PIN1, undergoing conformational changes and enhancing its transactivation activity towards the cyclin dependent kinase inhibitor, $p21^{waf1/cip1}$, potentially causing a transient G1 growth arrest. In order to investigate whether induction of $p21^{waf1/cip1}$ is strictly dependent on PIN1 interactions with p53, both p53 wildtype and null cell lines were tested for $p21^{waf1/cip1}$ induction using either specific PIN1 antisense oligonucleotides or exposure to AG122005, a small molecule PIN1 inhibitor (PPIase; Ki = 1.36 μM). The PIN1 antisense treatment resulted in 8 and 9-fold increases in $p21^{waf1/cip1}$ message and protein levels, respectively, in A549 cells. After 48 hours of AG122005 exposure, $p21^{waf1/cip1}$ protein levels were 4 and 5-fold induced in the p53 null cell lines, HT29 and CA46, respectively. When tested in p53 wildtype backgrounds, A549 and MCF-7 cells, $p21^{waf1/cip1}$ levels were induced to 2 and 6-fold of respective controls after exposure to AG122005. The same experiment was performed in an isogenic pair of HCT-116 cells transfected with the viral oncogene E6 which targets p53 for ubiquitinated mediated proteolysis. After 48 hours of exposure to AG122005, $p21^{waf1/cip1}$ was induced in both the p53 null-E6 background and the p53 wildtype-CMV background to 3 and 2-fold basal level, respectively. These results demonstrate that the PIN1 PPIase inhibitor, AG122005, is able to induce $p21^{waf1/cip1}$ independently of p53 status of the tumor cell. The lack of dependence on p53 for a known molecular mechanism of G1 arrest along with other demonstrated roles in cell cycle regulation and oncogenesis suggest the utility of PIN1 as a potential new drug target for treatment of human cancers.

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AICAR: a rational identified small molecule targeting Hsp90 chaperone function in cancer cells

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Background: The molecular chaperone heat shock protein 90 (Hsp90) is viewed as a "druggable" target for rational cancer therapy, due to its role at the crossroads of multiple signalling pathways associated with cell proliferation and cell viability. One Hsp90 client protein is survivin, an inhibitor of apoptosis protein (IAP) selectively overexpressed in most human tumors and involved in control of cell division and inhibition of apoptosis. In the present study, we used a structure- and dynamics-based computational design strategy to identify the non-peptidic small molecule 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) as a structurally novel inhibitor of Hsp90.
Material and Methods: The recently described peptidic antagonist of the survivin/Hsp90 complex, shepherdin [Plescia et al., Cancer Cell, 2005 (7)], was used as a scaffold to rationally identify low molecular weight compounds that may act as structurally novel Hsp90 antagonists, and a three-dimensional pharmacophore was built to screen a database of non-peptidic structures. ELISA was used to verify the specific binding of AICAR to Hsp90. To define the cellular effects of the drug, after a 72-h exposure to AICAR (31.25–250 μM), cells of different tumor cell types were assessed for growth potential, ability to undergo apoptosis and expression/activity of several Hsp90 client proteins.
Results: Experimental tests showed that AICAR binds the Hsp90 N-domain, destabilizes multiple Hsp90 client proteins *in vivo*, including survivin, AKT, CDK6 and telomerase, and exhibits dose-dependent antiproliferative activity in multiple tumor cell lines, while not affecting proliferation of normal human fibroblasts. Moreover, AICAR induced an apoptotic response in all tumor cell lines, as a consequence of the proteolytic activation of capase-9 and capase-3.
Conclusions: Based on these results, we propose that AICAR represents a viable lead for further development of novel Hsp90 antagonists structurally different from geldanamycins.

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Heat shock protein 27 down-regulation inhibits tumor progression and enhances gemzar chemotherapy in pancreatic cancer through activation of stat-3 signaling pathway

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Background: Despites the many advances in oncology over the last few decades, almost all patients with pancreatic cancer die of the disease. Significant progress in the understanding of important molecular processes associated with the development of the progression of the disease is helping tailor more effective treatment strategies. Molecularly targeted agents are offering hope for their potential role in helping translate the improved activity of combination chemotherapy into improved survival. Heat Shock protein 27 (Hsp27) is a chaperone implicated in several physiological tumor processes. Recently, a 2'-methoxyethyl modified phosphorothioate antisense oligonucleotide (OGX-427) that is complementary to Hsp27, inhibit Hsp27 expression and enhance drug efficacy in cancer xenograft model, has been developed. Phase I clinical trial using OGX-427 in patient with localized prostate cancer and high-risk features is starting in 2006 at the Prostate Centre (Vancouver). Our aim was to characterize changes in Hsp27 during pancreatic cancer outcome and assess the effects of OGX-427 on rates of pancreatic cancer apoptosis and tumour progression.
Materials and Methods: A tissue microarray was used to measure changes in Hsp27 protein expression in 150 specimen. Effects of OGX-427 on human pancreatic cancer MiaPaCa cell proliferation and apoptosis was assessed using the MTT assay and flow cytometer.
Results: Hsp27 expression was low or absent in differentiated tumors, but increased beginning in moderately differentiated tumors to become uniformly highly expressed in metastatic samples (>90%, $p < 0.01$). OGX-427 potently inhibited Hsp27 mRNA and protein expression (>70%) in MiaPaCa cells and resulted in >2 fold increases in the apoptotic subG0-G1 fraction and 75% of proliferation inhibition. Another mechanism mediating cytoprotection in pancreatic cancer involves stabilization with increased levels of stat-3, and we demonstrate down-regulation of total stat-3 protein levels and its activated genes after treatment with OGX-427. In vitro, Hsp27 down-regulation by OGX-427 increases Gemzar chemotherapy by 30% ($p < 0.01$). In vivo testing is in progress.
Conclusion: Increases in Hsp27 chaperone expression may serve a cytoprotective role in pancreatic cancer cells through mechanisms involving stat-3 signaling pathway activation. OGX-427 seems to be very potent to inhibit pancreatic cancer in vitro and deserves further study as a potential therapeutic.

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In vivo efficacy of BI 2536, a potent and selective inhibitor of the mitotic kinase Plk1, in combination with various cytotoxic agents

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Background: Polo-like kinase 1 (Plk1), a key regulator of cell cycle progression, represents an attractive target for cancer drug development as it is highly expressed in malignant cells and serves as a prognostic marker in certain human cancer types. We have previously shown that BI 2536, a potent and selective small-molecule inhibitor of Plk1, induces mitotic arrest and apoptosis in various human cancer models *in vitro* and *in vivo*. BI 2536, the first specific Plk1 inhibitor in clinical development, has demonstrated encouraging results in phase I trials. This study was designed to examine the efficacy of BI 2536 in combination with various established chemotherapeutic agents.
Methods: The human NSCLC model NCI-H460 was used to evaluate antitumour effects of BI 2536 in combination with docetaxel and cisplatin. For combination experiments with pemetrexed the human NSCLC model Calu-6 was utilized. The human colon carcinoma model HCT 116 was chosen to assess the antitumour effects of combinations of BI 2536 with irinotecan. Nude mice bearing subcutaneously human tumor xenografts were treated intravenously with suboptimal doses of BI 2536 or the above mentioned agents alone or in combination.
Results: In the NCI-H460 model the combination of BI 2536 (50 mg/kg i.v., once weekly) and docetaxel (15 mg/kg i.v., once weekly) showed clear antitumour efficacy with a T/C value of 26% whereas the single-agent treatments were less effective (T/C values of 65% and 42%, respectively). In the same model, a combination of BI 2536 (50 mg/kg i.v., twice weekly)