accumulation of autophagossomes (using fluorescent dyes) as well as of LC3-II (assessed by western blot), which was also significantly inhibited by SP. These results suggest that UA induction of apoptosis and autophagy is JNK dependent. A decrease in mutated p53 and phospho mTOR, which are associated with an induction of autophagy, were also observed. In conclusion, UA showed anticancer activity by inducing apoptosis and autophagy, which was JNK-dependent in HCT15 cells. In addition, in these resistant cells, UA synergistically cooperate with 5-FU to induce cell death.

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254 Down-regulation of P-gp and drug resistance to csiplatin and VP-16 in human lung cancer cell lines

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Background: The aim of this study is to investigate whether the expression of P-glycoprotein is correlated to chemotherapy resistance to cisplatin and VP-16 in four different subtypes of lung cancer cells.

Material and Methods: The expression of P-gp with or without pretreatment of verapamil in four different subtypes of lung cancer cell lines was analyzed with RT-PCR and immunofluorescence. Cell survival to cisplatin and VP-16 was determined by MTT assay.

Results: Our study shows that the expression of P-gp can be inhibited by verapamil in SPCA-1, NCI-H-460 and NCI-H-446 cell line, but not in SK-MES-1 cell line. With the pretreatment of verapamil, NCI-H-446 was more sensitiveto cisplatin (IC_{50}: 67.39±4.3 vs 50.69±2.25).; NCI-H-460, SPCA-1 and NCI-H-446 were more sensitive to VP-16 (IC_{50}: 67.39±4.3 vs 50.69±2.25, 62.37±2.88 vs 45.79±4.47 and 56.35±3.15 vs 43.61±1.64, respectively, $p < 0.05$) as well compared to the control group.

Conclusions: Verapamil can inhibit the expression of P-gp both at mRNA and protein level in NCI-H-460, SPCA-1 and NCI-H-446 lung cancer cell lines. The down-regulation of P-gp is associated with the intrinsic resistance to cisplatin in NCI-H-446 cell line and to VP-16 in NCI-H-460, SPCA-1 and NCI-H-446 cell lines. All these indicated that selecting appropriate mediator to inhibit the expression of P-gp maybe helpful for the reversion of drug resistance in some subtypes of lung cancer cell lines.

255 Withdrawn

256 A dichloromethane fraction of Strobilanthes crispus induces apoptosis and promotes the effect of tamoxifen in MCF-7 and MDA-MB-231 cells

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Background: The leaves of *Strobilanthes crispus* (*S. crispus*) which is native to the Malay Archipelago, are used in folk medicine for their antidiabetic, diuretic, anticancer and blood pressure lowering properties. Crude extracts of this plant have been found to be cytotoxic to cancer cell lines. In this study, an active fraction of the dichloromethane extract of *S. crispus* (SC/D-F9) was isolated and analysed for its anticancer activities in MCF-7 and MDA-MB-231 breast cancer cell lines.

Materials and Methods: The dichloromethane extract of *S. crispus* was chromatographed on a silica gel and SC/D-F9 was isolated by gradient step elution using a combination of hexane, DCM and MeOH. Cytotoxicity was measured using the LDH assay and apoptosis was determined using Annexin V antibody and analysed by flow cytometry and fluorescence microscopy. Alterations in the mitochondrial membrane potential were also determined by flow cytometry. Modulation of specific gene expression was determined using PCR array and analysed by real-time PCR.

Results: SC/D-F9 is relatively more cytotoxic to the MCF-7 and MDA-MB-231 cells compared to tamoxifen, paclitaxel and doxorubicin, while is non-cytotoxic to the normal breast epithelial cell line, MCF-10A. Cell death occurs by apoptosis via depolarization of the mitochondrial membrane potential and transcriptional modulation of specific signaling molecules. In addition, SC/D-F9 promotes the apoptotic effects of low dose tamoxifen on both breast cancer cell lines.

Conclusion: These findings suggest the potential of SC/D-F9 as a cancer therapeutic agent.

257 Effects of Drug-X on cisplatin-resistant and cisplatin-sensitive ovarian cancer cells

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Background: With 204,000 new cases and 125,000 deaths annually worldwide, ovarian cancer is the most lethal gynecological malignancy. Using current treatment options, recurrence is very common. The median survival rate for relapsing women is two to three years with only 30% surviving five years from diagnosis. Resistance to existing treatments (such as cisplatin) attributes to this poor survival rate. Consequently, our laboratory has identified a potentially new drug for treating ovarian cancer, Drug-X (name of the drug can not be disclosed at this point due to proprietary reasons). For decades, Drug-X has been widely used for other clinical indications. Herein, we investigated the effects of Drug-X on cisplatin-sensitive and cisplatin-resistant ovarian cancer cells.

Material and Methods: Three ovarian cancer cell lines (SKOV-3 [cisplatin-resistant], A2780-CP [cisplatin-resistant], and A2780 [cisplatin-sensitive]) were cultured and treated with Drug-X. To determine colony forming effects, 1000 cells were plated overnight, treated for 14 days, stained, and colonies were counted. To test mitochondrial effects, cells were treated (10−20 μM) at three different time points (6, 12, and 18 hours), incubated in 100 nM tetramethylrhodamine (TMRE) for 20 minutes, and analyzed by flow cytometry. In addition, after 48-hour treatment (1−27 μM), cells were analyzed using these methods: (1) Cell proliferation data was gathered by counting cells with an automated cell counter. (2) Apoptotic cells were identified using Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) and propidium iodide (PI) staining assays. (3) For immuno-blotting, cell lysates were prepared using RIPA buffer, electrophoretically resolved on SDS-PAGE, transferred to PVDF membrane, and probed with various apoptosis-related antibodies. Experiments were performed in triplicate, and statistical significance was determined using a two-tailed student t-test with equal variance.

Results: In all three cell lines, Drug-X inhibited cell proliferation and reduced colonogenic potential in a dose-dependent manner. Further analysis using TUNEL and PI staining revealed a dose-dependent increase in percent of apoptotic cells. Immuno-blotting assays showed a dose-dependent decrease in full-length caspase-9 and caspase-3 (indicating an increase in activity), and a marked increase in cleaved Poly (ADP-ribose) polymerase (PARP). The TMRE assay also revealed a dose-and time-dependent decrease in mitochondrial membrane potential ($\Delta\Psi m$) which is an early sign of the intrinsic apoptotic pathway.

Conclusions: Drug-X effectively inhibits growth of ovarian cancer cells *via* induction of caspase-mediated apoptosis. Our data suggest that Drug-X induces an intrinsic apoptotic pathway by altering mitochondrial membrane potential, triggering caspase-9 activity, and subsequently increasing caspase-3 activity and PARP cleavage. Therefore, Drug-X may be a potential treatment modality for cisplatin-resistant and cisplatin-sensitive ovarian cancer.

258 Anticancer activity of a novel kaempferol glucoside, Tac, is mediated by a mechanism involving RSK inhibition

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Background: The Ras/mitogen-activated protein kinase (MAPK) pathway regulates diverse cellular processes such as proliferation, survival, growth and motility. p90 ribosomal S6 kinase (RSK) constitutes a family of protein kinases downstream of the MAP kinase cascade and has been shown to regulate cancer progression by controlling cell proliferation. Recent studies on RSK's involvement in cancer pathways have shown that some flavonoids can act as specific inhibitors against members of the RSK family. In this regard, we have further investigated the mechanism of action of the semisynthetic kaempferol glusoside, named Tac, and whether this compound acts downstream to MAPK pathway via RSK inhibition. Finally, we have evaluated the antitumour activity of *Tac* in the human tumour xenograft/immunodeficient mouse model.

Materials and Methods: The antiproliferative effect of *Tac* was examined using the SRB assay. COMPARE algorithm was further employed for an initial evaluation of the mechanism of action. *Tac*-induced cell death perturbations on cell cycle were further examined on the HCT116 human colon cancer cell line using FACS analysis. Western blot was utilized for the detection of caspase activation, PARP cleavage and changes in the levels of MARCKS, p-MARCKS, ERK2, p-ERK1/2, RSK, p-RSK, total EF2, eEf2 and p-eEf2. The *in vivo* antitumour activity of *Tac* was also evaluated against HCT116 xenografts.

Results: COMPARE analysis revealed great similarities with DNA damaging agents. FACS analysis with PI stain indicated that *Tac* treatment resulted in