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Clusterin mediates chemoresistance in ovarian cancer cell lines

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Background: Clusterin (CLU) has been implicated in several physiologic processes as well as sensitivity to chemotherapy in many different types of cancer. Here we show the effect of paclitaxel (TX) on the expression and localization of CLU in Tx-sensitive and -resistant ovarian cancer cell lines.
Material and Methods: Ovarian cancer cells (KF) and their Tx-resistant counterpart (Kf-TX) were treated with paclitaxel in a dose and time course fashion and CLU expression was monitored by western blot. Confocal microscopy study and sub-cellular fractionation were performed to detect intracellular trafficking and distribution of CLU and quantify its different isoforms in response to TX treatment. Immunoprecipitation was performed to monitor the effect of TX treatment on the Ku70/CLU complex. Also, SiRNA targeting the secreted isoform of CLU was transfected into both sensitive and resistant cells and apoptotic fate was measured using FACS analysis and annexin-V staining.
Results: CLU expression was higher in Kf-TX than in KF. In Kf, CLU 60 KDa was up-regulated in response to TX treatment in a time and dose dependent manner, while different pattern were evident in KF-TX. Intracellular secretory sCLU, 40KDa, was up-regulated as an early event in both cells, but rapidly decreased with high doses of TX in KF cells, at difference with KF-TX. The same expression pattern was observed in the cell media. Confocal microscopy and sub-cellular fractionation studies revealed nuclear localization of intracellular (in) CLU in the sensitive cells but not in the resistant ones. Moreover, inCLU/Ku-70 complex accumulated in the nucleus in both cells after TX treatment but KF showed earlier nuclear accumulation than KF-TX. SiRNA transfection knocked down only the sCLU but not inCLU restored the sensitivity of KF-TX to TX treatment as confirmed by DNA ladder and FACS analysis.
Conclusion: Our data indicate a close relation between CLU intracellular trafficking isoforms and TX-sensitivity in ovarian cancer cells. Prevalence of nCLU accumulation appears to mediate TX-induced cell death, while sCLU might be protective. CLU gene products could be a novel target for a therapeutic molecule to enhance or restore chemo-sensitization of ovarian cancer cells.

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Role of MEK/ERK pathway in the MAD2-mediated cisplatin sensitivity in testicular germ cell tumour cells

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Background: Testicular germ cell tumour (TGCT) is the most common malignancy in young males. Cisplatin, a DNA damaging agent, is one of the most potent antitumour agents displaying clinical activity against a wide variety of solid tumours. Although most TGCTs are sensitive to cisplatin-based chemotherapy, significant numbers of TGCT patients still relapse and die each year because of the development of resistance to cisplatin. Previously, we first reported that a key regulator of the mitotic checkpoint, Mitotic Arrest Deficient 2 (MAD2), was a mediator of cisplatin sensitivity in human cancer cells. Also, recent research suggests that activation of MEK/ERK pathway plays an important role in cisplatin-induced cell death in TGCT cells. In this study, we investigated if MAD2 played a role in cellular sensitivity to cisplatin in TGCT cells and whether the MEK/ERK pathway was involved in this process.
Materials and Methods: We first studied an association between MAD2 expression and cisplatin sensitivity in 10 TGCT cell lines. The MAD2 level and cisplatin sensitivity were determined by Western blotting and colony forming assay respectively. The effect of cisplatin on the MEK/ERK pathway was identified by measuring the phosphorylation level of MEK1/2, ERK1/2 and Elk-1 proteins. To confirm the involvement of the MEK/ERK pathway in cisplatin-induced apoptosis, the effect of MEK/ERK inactivation on cisplatin sensitivity was examined by treating the cells with a MEK inhibitor, U0126. To examine the role of MAD2 in cisplatin sensitivity, a TGCT cell line expressing high level of MAD2 was transfected with a dominant negative MAD2 construct. The effect of MAD2 inactivation on the MEK/ERK pathway and cisplatin-induced apoptosis were studied by Western blotting, TUNEL assay and cell viability assay.
Results: Increased MAD2 expression was correlated with the cisplatin sensitivity in the TGCT cells. Also, inhibition of the MEK/ERK pathway resulted in protection of TGCT cells against cisplatin treatment. In addition, inactivation of MAD2 resulted in suppression of the MEK/ERK pathway and led to an increase in cisplatin resistance in TGCT cells.
Conclusion: Our data indicated that MAD2 expression was positively correlated with sensitivity to cisplatin and inactivation of MAD2 led to suppression of the MEK/ERK pathway, which resulted in resistance to cisplatin-induced cell death in TGCT cells. Our results suggested a novel mechanism responsible for cisplatin resistance in TGCT cells.

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Oral bioavailability and pharmacokinetics in CD2f1 mice of NSC73306, an antitumor agent that selectively kills multidrug-resistant cancer cells

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Background: NSC 73306 is a thiosemicarazone derivative with potent cytotoxic activity that is linked to P-glycoprotein mediated multidrug resistance (MDR1) activity, although the mechanism of action is not directly linked to interaction with P-glycoprotein [1]. The purpose of this study was to evaluate the pharmacokinetics of NSC 73306 in CD_2F_1 mice.
Methods: Positive electrospray ionization high performance liquid chromatography-tandem-mass spectrometry (LC-MS/MS) and MS^n analyses were performed using triple-quadrupole and ion-trap instruments, respectively, to identify characteristic fragment ions. Multiple-reaction monitoring of the most-abundant fragment ion was used for quantitation of NSC 73306 and the internal standard (one more methylene unit than the analyte). Short-term stability studies were carried out in human plasma and mouse plasma at 4ºC, 22ºC, and 37ºC, and long-term stability was analyzed at −20ºC in plasma. The pharmacokinetics of NSC 73306 in CD_2F_1 mice were determined after administration i.v. in a vehicle of ethanol/polyethylene glycol (PEG)/normal saline; p.o., formulated in dimethyl formamide/PEG; and i.p., formulated in 100% DMSO, at doses of 15 mg/kg, 60 mg/kg, and 100 mg/kg, respectively. Pharmacokinetic parameters were computed using WinNonlin computer software.
Results: NSC 73306 had a half life of approximately 9.5 h in mouse plasma at 37ºC and was slightly more stable in human plasma at this temperature. The half life at −20ºC was about 58 days in human plasma and 28 days in mouse plasma. After i.v. administration, the plasma concentration reached a mean concentration at 8.9 μM at 5 min, which declined biexponentially with time, with a mean $AUC_{0-\infty}$ of 3.67 h·μM. NSC 73306 had a distribution half life of 0.38 h and an elimination half life of 2.28 h following i.v. administration. The $AUC_{0-\infty}$ was 1.85 h·μM following p.o. administration, and 12.76 h·μM after i.p. administration at the doses indicated. Based on the AUC calculation, oral and i.p. availability were 12.6% and 52.1%, respectively. Less than 1% of the unchanged drug was recovered in 24 h urine after i.v. administration.
Conclusion: A sensitive and selective method for quantitating NSC 73306 in biofluids was developed and used to evaluate its pharmacokinetic behavior in CD_2F_1 mice. The results indicate that NSC 73306 has modest oral availability in mice.

References

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Role of DR5 and DcR1 in 5-FU apoptotic response of human colon carcinoma cells

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Background: Resistance to 5-fluorouracil (5-FU) has been frequently found in the treatment of digestive tract cancer patients. Since blockage in apoptosis induction is accepted as one of the mechanisms responsible for this resistance, the use of the death receptors (DRs) ligand as potent therapeutic agent has emerged. Induction of apoptosis in tumor cells by TNF-related apoptosis-inducing ligand (TRAIL) is believed to be regulated by expression of two death-inducing (DR4 and DR5) and two inhibitory or decoy receptors (DcR1 and DcR2) on the cell surface. In this study, we addressed the role of the death receptors-dependant pathway by comparing the 5-FU-sensitive HCT116 cells with its derivative resistant D59 colon carcinoma cells.
Methods: 5-FU sensitivity assays were determined using Sulforhodamine-B assay to evaluated IC50 values. TRAIL and/or 5-FU induced apoptosis was revealed by AnnexinV and propidium iodide staining. To determinate the involvement of the TRAIL receptors expression on the colon cancer cells, we used real time PCR, flow cytometry and immunoblot analysis.
Results: The IC50 values for 5-FU were determined to be respectively 10 μM and 100 μM for the sensitive and resistant cells using 24 hours cytotoxicity assay. Parental cells had a highest population of apoptotic