Intraspecies hybrids of the sensitive and resistant KB cells (single step: KCP.5 resistant to 0.5 mg/mL of cisplatin, two steps: KCP1 resistant to 1 mg/mL of cisplatin) were fused with D98OR (HAT sensitive, ouabain resistant) to determine whether cisplatin resistance is a dominant or recessive trait. Cell-cell hybridization between the sensitive cells and single-step or two-step KB cisplatin resistant cells both indicated codominance of cisplatin resistance compared to hybrids between sensitive cell lines (D98ORxKB). The hybrids between sensitive cell lines (D98xKB) and a single-step cisplatin resistant KB cell line (D98xKCP.5) also were cross-resistant to carboplatin. In addition, based on the doubling times of hybrid cells, the relatively slower growth rate of cisplatin resistant cells appears to be dominant. Previous work in our lab has found membrane protein mislocalization in the KB cisplatin resistant cell lines, including intracellular accumulation of folate binding protein (FBP). Confocal microscopy imaging of the D98xKCP.5 hybrids for FBP showed the same mislocalization as the parental cisplatin resistant cell lines, further indicating that mislocalization of FBP is likely to be a dominant phenotype linked with cisplatin resistance, consistent with a molecular defect in inability of cisplatin binding/transport proteins to get to the cell surface. In the two-step cisplatin resistant KB cell line, KCP1, resistance is no more dominant than in the single-step cisplatin resistant KB cell line, KCP.5, suggesting that one of the two steps of resistance in KCP1 may not be dominant. These dominance data suggest that it might be possible to identify a gene or genes responsible for cisplatin resistance by gene transfer from a resistant cell line in a sensitive cell line.

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DNA hypermethylation and resistance to chemotherapy in ovarian cancer

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Aberrant DNA methylation is one of the hallmarks of tumours. Human cancers show altered patterns of CpG island (CGI) methylation at genes involved in essentially every facet of tumour development. We have shown that CGIs associated with genes known to be involved in drug sensitivity, such as hMLH1, can become methylated in ovarian tumours and that treatment of resistant cells with DNA methyltransferase (DNMT) inhibitors can sensitise tumour cells to a variety of cytotoxic chemotherapeutic drugs. In a pilot study we have identified patterns of increased gene methylation, using array-based differential methylation hybridisation (DMH), which predict poor progression-free survival in ovarian cancer (Wei et al 2002, Clin. Cancer Res., in press). Thus, ovarian tumours with such increased CGI methylation may help define patient populations for combination treatment of DNMT inhibitors with cytotoxics such as carboplatin. The MCJ gene, a member of the DNAJ (HSP40) protein superfamily, has been identified as a target for aberrant methylation and shown to play a role in sensitivity to cisplatin (Shridhar et al 2001, Cancer Res. 61:4258). We show that expression of the MCJ gene is lost in 8/10 cisplatin-resistant derivatives of the ovarian carcinoma cell line A2780. Furthermore, treatment of two of the resistant cell lines with the DNMT inhibitor 5-azacytidine, resulted in re-expression of MCJ, suggesting that loss of expression may be due to increased methylation. A CGI was identified beginning 164bp downstream of the transcriptional start site, within the first exon of the MCJ gene. Bisulfite sequencing of this region in normal ovarian tissue DNA determined that about 50% of clones were densely methylated and about 50% of clones largely unmethylated. MCJ expressing cell lines revealed a pattern of methylation similar to normal DNA. However, the cisplatin-resistant, non-expressing, cell lines exhibited dense methylation of 100% of clones sequenced. These results suggest that methylation of the intragenic CGI of MCJ can result in loss of gene expression. Sequencing of this region of the MCJ gene in a cohort of 32 ovarian tumour samples identified a subset of tumours (16%) that exhibited high levels of methylated clones (>90%). Furthermore this identified a possible link between high levels of MCJ methylation and poor response to chemotherapy following platinum based chemotherapy in ovarian cancer patients ($p=0.01$).

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Preclinical rationale for a combined treatment with irinotecan and the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) ZD1839 (Iressa) in irinotecan-refractory human colon cancer

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EGFR over-expression and activation of its intrinsic TK are involved in malignant transformation, which is commonly associated with poor clinical prognosis. EGFR targeting agents have shown antiproliferative activity in clinical trials, eg monoclonal antibody IMC-C225 (cetuximab) in patients (pts) with irinotecan-refractory colorectal cancer (CRC) [ASCO 2001, abs 7] or small molecule EGFR-TKIs, eg ZD1839 (Iressa), in NSCLC (ASCO 2002, abs 1166, 1188). We investigated the effect of ZD1839 on cellular determinants of resistance to the active metabolite of irinotecan (SN-38) in drug-sensitive (HCT-8/wt) and resistant (HCT-8/SN-38) human colon cancer cells. Co-administration of ZD1839 at non-cytotoxic concentrations completely restored sensitivity to SN-38 in EGFR-expressing HCT-8/SN-38 cells, both in the presence or absence of EGF (1-100 ng/mL). ZD1839 did not affect topoisomerase I (Topo I), Topo II-b and general protein expression, but we observed a significant time- and dose-dependent downregulation of Topo II-a protein and inhibition of its enzymatic function, which corresponded to a G1-phase block in cell cycle analyses. These results were confirmed using quantitative RT-PCR. ZD1839 dose-dependently increased SN-38-mediated induction of protein-linked DNA single-strand breaks (for 10 μM SN-38: 5.1 $\pm$ 0.87% [IC_{50} ZD1839] and 15.9 $\pm$ 0.44% [4-fold IC_{50} ZD1839] vs 2.42 $\pm$ 0.29% [untreated] [$p=0.005$ and $p<0.0001$, respectively]), with no alteration of Topo I protein expression or unwinding activity of pBR322 plasmid DNA using nuclear extracts of HCT-8/SN-38. Neither induction of resistance to SN-38 nor the following exposure to ZD1839 showed an influence on EGFR expression, but there was a significant decrease in EGFR phosphorylation levels with ZD1839 in specific immunoblotting and immunocytochemical analyses. Cellular pharmacokinetics of the active SN-38 lactone revealed no significant differences of drug accumulation or retention by ZD1839 using HPLC. Analyses of membrane transporters, EGFR downstream signaling and differential gene expression in the resistant cell line with or without ZD1839 treatment will be presented. In conclusion, inhibiting EGFR-TK activation with ZD1839 reverses resistance to SN-38 in human colon cancer cells. These data support combination therapy with ZD1839 and irinotecan in patients with CRC that is refractory to irinotecan-based regimens. Iressa is a trademark of the AstraZeneca group of companies

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Pharmacogenetics of the human glutathione S-transferase P1 gene and tumor response to chemotherapy

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The polymorphic human GSTP1 gene locus encodes proteins that differ functionally in their metabolism of electrophilic compounds, including a number of anticancer agents. This study was designed to gain a better insight into the potential role of the GSTP1 genetic polymorphism in the outcome of cancer chemotherapy. Using a prokaryotic system and GSTP1-null human tumor cells genetically modified to express each of the GSTP1 alleles, we investigated the differential protection conferred by the GSTP1 alleles against four anticancer agents, namely, carboplatin, cisplatin, thiotepa, and 4-hydroperoxyifosfamide (HI). In the prokaryotic system, E. coli were transformed with expression vectors carrying cDNAs of the GSTP1 alleles and the cytoprotective effects examined in a clonogenic survival assay. Simultaneously, isogenic variants of the GSTP1-null human medulloblastoma cell line, engineered to stably express each of the GSTP1 alleles, were examined for altered resistance to cisplatin, and for the level of cisplatin-induced DNA damage and apoptosis. The results showed all GSTP1 alleles to be cytoprotective against the anticancer agents. For cisplatin and carboplatin, the GSTP1*C allele was most protective followed by GSTP1*B and GSTP1*A. In contrast, protection against thiotepa was highest for GSTP1*A followed by GSTP1*B and GSTP1*C. Protection against 4-HI was the same for both GSTP1*B and GSTP1*C and higher than GSTP1*A. In the medulloblastoma cells, the levels of cisplatin-induced DNA damage and apoptosis were decreased by all three GSTP1 alleles in the order GSTP1*C > GSTP1*B > GSTP1*A the same order as was observed for the increase in cisplatin resistance. Using HPLC and mass spectral anal-