ysis, we showed that formation of the mono-glutathionyl platinum metabolite was catalyzed differentially by the GSTP1 allelic proteins, the highest levels being with GSTP1*C followed by GSTP1*B and GSTP1*A. These results, along with the results of molecular docking studies of thiotepa and 4-HI in the active sites of the GSTP1 allelic proteins, suggest that the GSTP1 alleles differ in their ability to protect cells against electrophilic anticancer agents, and that this results, at least in part, from differences between the GSTP1 proteins to metabolize anticancer agents. A GSTP1 genotype-based differential protection of tumor cells against the cytotoxicity of anticancer agents may thus be an important pharmacogenetic determinant of clinical response to cancer chemotherapy.

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Functional cloning of drug resistance genes using retroviral cDNA expression libraries

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To improve the curative success of chemotherapy, it will be essential to understand the molecular basis of drug resistance (DR). The availability of high-complexity retroviral cDNA libraries enables "large-scale" genetic screens for DR genes by phenotypic selection following random gene overexpression. We have developed a cell culture system that enables the functional cloning of mammalian DR genes by this approach, and here we show proof of principle of our system using the anticancer drug cisplatin. Retroviral packaging cells were transfected with a human placenta retroviral cDNA library, which led to the production of high-titer, replication-deficient virus containing the unknown cDNAs. Viral supernatant was used to infect excision repair cross complementation group 1 (ERCC1)-deficient, and therefore cisplatin-hypersensitive mouse embryonic fibroblast (MEF) target cells. After selection with cisplatin, 24 primary DR cell clones were picked and grown separately. To confirm that the DR phenotype resulted from the expression of an integrated retroviral cDNA insert (provirus), the primary clones were infected with a wild-type murine Moloney virus to mobilize the proviruses. These proviruses were subsequently produced as infectious particles into the culture medium, which was then used to infect fresh target cells prior to a second round of cisplatin selection. The cisplatin resistance of 18 primary DR clones could be confirmed. PCR and subsequent sequencing revealed that each of these clones was rescued from drug-induced cell death by a recurring ERCC1 gene from the cDNA library. Surprisingly, 9 of these clones contained 5'-truncated ERCC1 sequences in which the reported ATG start codon is absent. The resulting protein, when encoded from the first subsequent in-frame ATG codon, should be inactive since it lacks the binding domain for the damage recognition protein XPA that is required for ERCC1 repair activity. Notably, the XPA binding domain sequence was present in the truncated cDNAs, and must have been translated to result in functional ERCC1 protein. We conclude that our culture system enables phenotypic selection of genes whose expression compromises cisplatin-induced cell death. Moreover, translation of 5'-truncated cDNA inserts can start upstream on the vector sequence, improving the chances of success using these retroviral cDNA libraries.

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The mTOR inhibitor, CCI-779, restores tamoxifen response in breast cancer cells with high Akt activity

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The Akt kinase is a serine/threonine protein kinase that has been implicated in mediating a variety of biological responses. Studies show that high Akt activity in breast carcinoma is associated with a poor pathophenotype as well as hormone and chemotherapy resistance. Additionally, high Akt activity is associated with standard markers for a poor outcome prognosis. Thus, a chemotherapeutic agent directed specifically towards tumors with high Akt activity could prove extremely potent in treating those breast tumors with the most aggressive phenotypes. Several studies have demonstrated that rapamycin, which inhibits mTOR, a downstream target of Akt, sensitizes certain resistant cancer cells to chemotherapeutic agents. We are currently evaluating the efficacy of mTOR inhibition in the treatment of tamoxifen-resistant breast carcinoma characterized by high Akt activity. We found that MCF-7 breast cancer cell lines expressing a constitutively active Akt are able to proliferate under reduced estrogen conditions, and are resistant to the growth inhibitory effects of tamoxifen, both *in vitro* as well as *in vivo* in xenograft models. Co-treatment with rapamycin *in vitro*, or the structural analog of rapamycin, CCI-779 (Wyeth-Ayerst) *in vivo*, restored sensitivity to tamoxifen. The average TGI of each tumor type with CCI-779 alone, tamoxifen alone, or with the combination of CCI-779 and tamoxifen is as follows:

	Control MCF-7 Tumors	Akt MCF-7 Tumors
CCI-779	21%	42%
Tamoxifen	64%	28%
CCI-779 and Tamoxifen	62%	76%

Molecular analyses to determine alterations in signaling transduction pathways, apoptotic and proliferative responses are on going. These data corroborate prior findings indicating that Akt activation induces resistance to tamoxifen in breast cancer cells. Importantly, inhibition of the PI3K/Akt pathway by CCI-779 restores the susceptibility of these cells to tamoxifen. These data may have implication for future clinical studies of CCI-779 in breast cancer cells.

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Gene expression profile of cisplatin resistance in ovarian cancer cell lines

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Cisplatin resistance continues to be a major obstacle in the successful treatment of advanced ovarian cancer. There is a diverse set of mechanisms that have been shown to play a role in acquired resistance, ranging from cellular uptake and efflux, intracellular sequestration of cisplatin to differences in the response and repair pathways induced by DNA damage. The goal of our study was to use an *in vitro* model of cisplatin resistance and compare the expression profiles of four derived, increasingly resistant cell lines to a common parental sensitive cell line, as well as to compare their response following cisplatin exposure, using a 15K cDNA microarray. First, we compared the expression profile of the cisplatin sensitive and resistant cell lines at baseline (prior to drug exposure) and identified genes that showed the most significant difference. We correlated these findings with two other cell lines with known intrinsic properties of cisplatin resistance and sensitivity (SKOV3, OVCAR4). Our results showed 70 genes with significant difference in expression between the resistant cells and the reference (sensitive) cell line. One fifth of these genes correlated with the expression pattern of the other two cell lines with known intrinsic cisplatin resistance. We also compared the gene expression patterns of the two cell populations following induction by cisplatin. Using clustering algorithms we identified genes that appeared to be differentially regulated between the two cell lines. Among these genes are several known cell cycle regulators, pro-apoptotic genes and DNA damage repair genes, as well as new potential candidates of these pathways. Our study provides the expression profile of an *in vitro* model of cisplatin resistance before and after cisplatin induction and allows us to identify genes that could distinguish the sensitive and resistant phenotype.

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Novel splice variants in the ABCC1 (multidrug resistance-associated protein-1) gene in ovarian cancer

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Ovarian cancer strikes more than 23,000 American women annually, with ~14,000annual deaths. While the past decade has brought new understanding of theetiology of this disease, treatment advances have not kept pace, and women stillsuccumb to therapy-resistant disease. While resistance associated withoverexpression of P-glycoprotein (the product of the ABCB1/MDR1 gene) has beendocumented in breast and other cancers, this is not usually seen in ovariancancer. Of interest, the expression of the related gene family member,multidrug resistance-associated protein (Mrp)-1, the product of the ABCC1/MRP1gene, is more ubiquitous, and while its