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.

527

Functional cloning of drug resistance genes using retroviral cDNA expression libraries

P.T. Bosma[1], A.W.M. Boersma[1], R. Oostrum[1], N.G.J. Jaspers[2], G. Stoter[3], K. Nooter[1]. *[1]Erasmus MC - Josephine Nefkens Institute, Medical Oncology, Rotterdam, The Netherlands; [2]Erasmus MC - Medical Genetics Cluster, Cell Biology and Genetics, Rotterdam, The Netherlands; [3]Erasmus MC - Daniel den Hoed Cancer Center, Medical Oncology, Rotterdam, The Netherlands*

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.

528

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

L. deGraffenried[1], W. Friedrichs[1], L. Fulcher[1], J. Silva[1], R. Roth[2], M. Hidalgo[3]. *[1]UT Health Science Center at San Antonio, Medical Oncology, San Antonio, USA; [2]Stanford University School of Medicine, Dept of Molecular Pharmacology, Stanford, USA; [3]Johns Hopkins Medical Center, The Johns Hopkins Oncology Center, Baltimore, USA*

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.

529

Gene expression profile of cisplatin resistance in ovarian cancer cell lines

T. Gonda[1,2], R. Walker[1], A. Sood[3], M. Hendrix[3], P. Meltzer[3]. *[1]NHGRI, NIH, Bethesda, USA; [2]NIH-HHMI Research scholars Program, Howard-Hughes Medical Institute, Bethesda, USA; [3]Department of Gynecology, University of Iowa, Iowa City, USA*

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.

530

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

W. Beck, X. He, R. Ee, Y. Mo. *University of Illinois at Chicago, Biopharmaceutical Sciences, Chicago, USA*

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

expression can confer resistance to multiple natural product drugs, it has not been shown to be associated with thechemotherapeutic resistance of ovarian cancer. Using anonymized specimens fromthe Gynecologic Oncology Group (GOG) Tumor Bank, we have observed that the ABCC1 gene is overexpressed in pre-treatment ovarian cancer, compared to matched-normal ovarian epithelium, and we have identified a number of splice variants ofthis gene that appear to be uniquely expressed in these tumor specimens,suggesting that they may play some role in the therapeutic insensitivity ofovarian cancer. In support of this idea, we have also seen such splice variantsin a leukemic cell line selected for resistance to teniposide, andcross-resistant to etoposide, a drug used to treat ovarian cancer. (Supportedin part by grants from the National Cancer Institute [to WTB] and in part by theGOG [the Core Lab in Molecular Pharmacology, to WTB])

531

AKT as a target for enhancing breast cancer chemotherapy and radiotherapy

Z. Fan[1], L. Ke[1], C. Knuefermann[1], M. Schmidt[1], W. Jin[1], Y. Lu[1], K. Ang[2], L. Milas[3]. [1]*Experimental Therapeutics,* [2]*Radiation Oncology,* [3]*Experimental Radiation Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, USA*

The phosphatidylinositol-3 kinase (PI-3K) pathway, regulated by its upstream oncoproteins such as the growth factor receptors with tyrosine kinase activity or Ras, play a critical role in promoting cancer cell proliferation and inhibiting cancer cell death. Akt, the cellular homologue of the viral oncogene v-akt, is an important mediator of such effects of the PI-3K. However, it has not been established whether increased activity of Akt could directly render breast cancer cell resistance to chemotherapy or radiotherapy. In this study, we demonstrated a causal role of Akt in conferring resistance to chemotherapy- and radiotherapy-induced apoptosis on MCF7 human breast cells. MCF7 cells were stably transfected with a farnesylated Akt expression vector, which we demonstrated was constitutively active. We assessed the effect of the farnesylated Akt on the sensitivity of MCF7 cells to several chemotherapeutic agents that are currently used for breast cancer patients, and on the sensitivity of MCF7 cells to radiotherapy as well. Compared with control vector-transfected MCF7 cells, MCF7 cells expressing farnesylated Akt (MCF7Akt-farn) showed significantly greater resistance to the cytotoxic effects mediated by paclitaxel, doxorubicin, etoposide, 5-fluorouracil, or camptothecin, and showed a markedly increased clonogenic survival rate following 5 Gy irradiation (from 7.3% in MCF7 control vector-transfected cells to 16.9% in the constitutively active MCF7Akt-farn cells). We next examined the effects of inhibiting the PI-3K pathway with the specific inhibitor LY294002 on MCF7 cells that were stably transfected with HER2 or the constitutively active RasG12V mutant; both showed PI-3K-dependent (LY294002-sensitive) increase in Akt activity. Compared with control vector transfected cells, MCF7HER2 or MCF7RasG12V cells showed increased resistance to these chemotherapeutic agents and to gamma irradiation. Co-treatment of these MCF7HER2 or MCF7RasG12V cells with LY294002 markedly inhibited Akt activity, and sensitized these transfectant cells to the treatment with chemotherapeutic agents or with radiotherapy. Our results suggest that Akt plays an important role in conferring resistance to conventional chemotherapy and radiotherapy on breast cancer cells and therefore may be a target for improving the therapeutic outcome of breast cancer treatments.

532

Rapamycin, an inhibitor of mTOR, reverses chemoresistance in PTEN negative prostate cancer xenografts

V. Gruenwald, M. Hidalgo. *The Sidney Kimmel Comprehensive Cancer Center at J, Experimental Therapeutics, Baltimore, USA*

PTEN is a lipid phosphatase with tumor suppressing abilities, which is frequently mutated or deleted in many different cancers. Loss of PTEN leads to activation of the PI3k/Akt pathway, which promotes cellular survival and has been associated with chemoresistance. We showed in previous studies that resistance to doxorubicin in prostate cancer cells is conferred by loss of PTEN/activation of Akt, and that the mTOR inhibitor rapamycin reverses this chemoresistance upon co-treatment in PTEN negative prostate cancer cells (Proc. Am. Assoc. Cancer Res. 2002; vol. 43: Abstract 4703). In the current study we aimed to determine the *in vivo* effects of rapamycin on response to treatment with doxorubicin in the PTEN negative prostate cancer cell line PC-3. Nudemice were inoculated with PC-3 xenografts and treated when tumors reached 200 mm3 with CCI-779 (10 mg/kg d1-5 i.p.), an ester derivative of rapamycin currently in clinical development, doxorubicin (10 mg/kg d1 i.v.), or a combination of both compounds at the same dose levels and schedules. Response data are now available at 2 weeks of follow up. So far, doxorubicin achieved a tumorstatic effect, whereas CCI-779 showed a 40% tumor reduction, and the combination therapy yielded a 50% decrease in tumor volume. Tumor growth reoccurred for mice treated with CCI-779 alone on day 11, whereas tumor volumes for the combination therapy arm remain low. The study is still ongoing and complete data will be presented at the meeting, including molecular analyses of effectors of the PI3k/Akt pathway of the treated tumors. This encouraging data will expectantly provide the rational to explore in clinical trials whether CCI-779 increases the response to chemotherapy of patients with PTEN negative/Akt active prostate cancers.

533

JNK and p38 MAP kinases potentially contribute to tamoxifen resistance of breast cancer via direct phosphorylation of both estrogen receptor and AIB1 coactivator

J. Shou[1,2], J. Wong[2], N. Weigel[2], C. Osborne[1,2], R. Schiff[1,2]. [1]*Baylor College of Medicine, Breast Center, Houston, USA;* [2]*Baylor College of Medicine, Dept. of Molecular and Cellular Biology, Houston, USA*

De novo and acquired endocrine resistance is a major clinical problem in management of breast cancer. Using an *in vivo* xenograft model of breast cancer endocrine resistance, we have recently shown that the development of resistance to tamoxifen (TamR) and to prolonged estrogen withdrawal (-E2R) is associated with cellular stress and increased levels of the stress-related kinases JNK and p38 MAPK. Increased JNK has also been documented in clinical TamR tumors. We therefore hypothesize that JNK and p38 MAP kinases are important determinants in the process of acquiring resistance, presumably through activation of the estrogen receptor (ER) pathway by phosphorylation of both ER and its coactivators. We found, using *in vitro* kinase assays, that all forms of p38 MAPK (alpha, beta, gamma and delta) phosphorylate both ER alpha and ER beta, and that the p38 alpha- and beta-induced phosphorylation of ER can be inhibited by the p38 specific inhibitor SB203580. Using truncated mutants of ER alpha we found that p38 phosphorylates the AF1 domain of the receptor. The ER coactivator AIB1 is often amplified and overexpressed in breast tumors, and we have recently found that AIB1 is an important component of TamR found in Her2-overexpressing tumors. Interestingly, we found that both JNK and p38 MAPK can directly phosphorylate AIB1 *in vitro*. Thus, as has been suggested for growth factor signaling, AIB1 may also be a conduit for kinase-mediated stress signaling to the ER pathway. Our data suggests that increased active JNK and p38, and cross-talk between these pathways and the ER pathway, may play a key role in endocrine resistance through phosphorylation and activation of different components of the ER pathway. We are currently studying whether specific JNK and p38 inhibitors can circumvent endocrine resistance *in vivo* in our xenograft breast cancer model.

534

Oligonucleotide chip analysis reveals distinctive gene expression patterns in Tam-sensitive and -resistant human mammary carcinoma xenografts

M. Becker[1], A. Sommer[2], J. Krätzschmar[2], H. Seidel[2], H.-D. Pohlenz[2], I. Fichtner[1]. [1]*Max Delbrueck Center for Molecular Medicine, Experimental Pharmacology, Berlin, Germany;* [2]*Schering AG, Research Laboratories, Berlin, Germany*

Aim of study: The mechanisms by which human mammary tumors fail to respond to Tamoxifen (Tam) therapy are mainly unknown. We undertook a comparative gene expression analysis of a Tam-sensitive and -resistant human breast cancer *in vivo*-model to identify molecular targets being involved in Tam resistance.
Methods: Originating from a Tam-sensitive human mammary carcinoma xenograft (MaCa 3366) we successfully established the Tam-resistant model MaCa 3366/TAM by treatment of tumor-bearing nude mice with Tam for 3 years during routine passaging. Samples from both tumor lines were used for comparative analysis. The 5 treatment groups were: MaCa 3366 and MaCa 3366/TAM each supplemented with E2, with E2 plus short-term treatment with TAM, and MaCa 3366/TAM under permanent Tam treatment. Total RNA from the tumor tissues was pooled per group and hybridized to Affymetrix HuGeneFL chips interrogating approximately 7000 human genes and ESTs. Pairwise comparisons and clustering algorithms were used to identify differentially expressed genes and patterns of gene expression, respectively.