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])

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AKT as a target for enhancing breast cancer chemotherapy and radiotherapy

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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.

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

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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.

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

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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.

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

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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.