etic/erythropoietic cell ratio. Pharmacokinetic parameters were obtained for each dose level after the first dose, first cycle and last dose, last cycle and indicate dose-proportional drug exposure of all treated animals.

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Lipid rafts as gateway for antitumor alkyl-lysophospholipids to induce apoptosis

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Synthetic alkyl-lysophospholipids (ALPs), such as 1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine, are antitumor agents known to accumulate in cell membranes. The aim of this study was to understand the mechanism by which ALP enters the cell and induces apoptosis. We demonstrate that in murine lymphoma S49 cells, ALP inhibits de novo biosynthesis of phosphatidylcholine (PC) at the CTP:phosphocholine cytidylyltransferase (CT) step. Exogenous lysoPC providing an alternative route to generate PC (via acylation), rescued the cells from ALP-induced apoptosis. This indicates that a continuous rapid PC turnover is essential for cell survival. To reach CT, ALP needs to be internalized. This internalization did not involve receptor/clathrin-coated pit-mediated endocytosis, nor fluid phase endocytosis. Instead, intact lipid rafts in the plasma membrane were found essential, as ALP was found to accumulate in lipid rafts and artificial disruption of these microdomains resulted in dissociation of ALP from rafts. This led to a reduced ALP endocytosis, and inhibition of apoptosis. Interestingly, an ALP-resistant cell variant, S49AR, showed no impaired PC metabolism after ALP treatment and revealed reduced ALP internalization and reduced levels of sphingomyelin, an essential component of lipid rafts. Therefore, we argue that altered lipid composition of lipid rafts determine raft-mediated endocytosis. For the first time, lipid rafts are recognized as potential targets for anticancer therapy.

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NADPH oxidase 1 (NOX 1): a novel target for colon cancer therapy

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Recent studies have demonstrated the presence of several novel membrane oxidases in mammalian tissues that share homology with gp91phox, the catalytic moiety of the NADPH oxidase (NOX) found in phagocytic leukocytes. These flavoproteins catalyze the NADPH-dependent reduction of oxygen to superoxide and related reactive oxygen species (ROS). Because the mechanism of ROS generation after exposure of human tumor cells to a wide range of growth factors (including EGF, PDGF, insulin, bFGF, and GM-CSF) remains to be determined, we examined the expression of NOX 1 in a panel of cultured human cancer cells using real-time RT-PCR. NOX 1 mRNA was quantitated as the ratio of the levels of NOX1/18S mRNA using specific plasmids containing either NOX1 or 18S. NOX 1 expression ratios were very high (>130,000) in human colon cancer cell lines (CaCo2, LS174T, and HT-29) and barely detectable (ratios <200) in human MDA-MB468, BT474, and ZR-75 breast cancer cells or DU-145 and LNCap human prostate cancer lines. In a panel of twelve human colon cancers paired with adjacent normal tissues obtained from the City of Hope Frozen Tumor Bank, NOX 1 ratios ranged from 20,000 to 800,000 in 10/12 tumors, were undetectable in 2/12 tumor and normal samples, and were substantially greater in tumor than normal tissue in 7/10 samples. As was the case in cell lines, NOX 1 expression ratios were low (<500) in 12/12 human breast cancer specimens and 6/6 prostate cancers. To assess NOX 1 as a therapeutic target, growth inhibition by the NOX inhibitor diphenylene iodonium (DPI) was examined *in vitro*. The IC_{50}'s for DPI were 5, 20, and 40 nM for CaCo2, LS174T, and HT-29 cells that express high levels of NOX 1, and were >2000 nM for DU-145 and MDA-MB468 cells that demonstrate very low level expression of the oxidase. These experiments suggest that growth factor-related reactive oxygen production may play an important role in signal transduction and tumor cell proliferation; and that NOX 1 may be a new target for the development of novel treatments for colon cancer. (Supported by CA 62505)

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Molecular modes of action of antimalarial artemisinin derivatives as novel anticancer drugs

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Twenty-two chemically characterized compounds derived from Traditional Chinese Medicine were analyzed in drug-sensitive and multidrug-resistant tumor cell lines. The antimalarial artesunate (ART), a semisynthetic derivative of artemisinin from the Chinese plant *Artemisia annua L.*, was among the most active compounds. ART did not exhibit cross-resistance to multidrug-resistant tumor cells overexpressing either the resistance-conferring MDR1, MRP1, or BCRP genes. Isogenic p53 -/- knock out tumor cells were as sensitive as their p53+/+ counterparts indicating that ART was not subject to p53-mediated chemoresistance. The evaluation of ART's anticancer activity in 55 cell lines of the National Cancer Institute, U.S.A., showed that ART was most active against leukemia and colon cancer cell lines. We mined the N.C.I. database and correlated the IC_{50} values with microarray mRNA expression profile of 464 genes. By hierarchical cluster analysis we identified oncogenes and proliferation-regulating genes which were strongly downregulated in ART-sensitive leukemia and colon cancer cell lines. The role of proliferation for ART's response was corroborated using a panel of *Saccharomyces cerevisiae* strains with defined genetic knock out mutations. Furthermore, ART correlated significantly with proliferation parameters (cell doubling times, G0/G1 and S cell cycle phases). We extended our analyses to other artemisinin derivatives, arteether and artemether. Using hierarchical cluster analysis we found that one cluster of genes correlated with the IC_{50} values of all three derivatives, the majority of them being proliferation-associated genes. This speaks again for a general role of the proliferative state for the response of tumor cells towards artemisinin derivatives. Another cluster contained genes correlating specifically with one of the 3 drugs. The correlation to different genes may explain differing anticancer activities of artemisinins.

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Blockade of endothelin A receptor by ABT 627 suppresses tumor growth, neovascularization and potentiates cytotoxic paclitaxel activity in ovarian cancer cells *in vitro* and *in vivo*

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The endothelin-1 (ET-1)/ETA receptor (ETAR) autocrine pathway is overexpressed in many human tumors, including ovarian carcinoma, and may provide a new target for anticancer therapy. Engagement of ETAR by ET-1 triggers activation of tumor cell proliferation, survival, neoangiogenesis and invasion. In primary and metastatic ovarian carcinomas, ET-1 overexpression is associated with enhanced neovascularization as well as with vascular endothelial growth factor (VEGF) expression. ABT627 (Atrasentan) is a p.o.-active ETAR antagonist that selectively inhibits the ETAR activities and is under clinical develoment in cancer patients. We therefore tested whether ABT627 may potentially block ovarian tumor progression and may affect neovascularization and apoptosis. When tested in culture ABT627, inhibited tumor growth in both primary cultures (PMOV1 and PMOV2) and cell lines (OVCA 433 and HEY) of ovarian carcinoma. In contrast, the ETBR antagonist, BQ 788, does not display inhibitory effects. Furthermore ABT627 inhibited VEGF production and enhanced proapoptotic effect of paclitaxel. Extending these studies *in vivo*, we explored the therapeutic effects of ABT627 on HEY ovarian carcinoma xenografts. HEY cells produced high amount of ET-1, expressed high affinity ETAR (Kd=0.1 nM; 35,600 sites/cell) and developed rapidly growing solid tumors in nude mice. ABT627 (2mg/Kg/24h i. p. for 21 days) produced similar inhibition of tumor growth as paclitaxel (20mg/Kg i. v. Q4x3) with a reduction of 65% (p=0.005) and 67% (p=0.006), respectively, compared with control. Similar results were obtained with high dosage of ABT 627 (10mg/Kg/24h). Immunohistochemical evaluation of tumors revealed that the reduced size of ABT627-treated tumor xenografts coincided with reduced neovascularization and with enhanced ovarian cancer cell death. Administration of ABT627 and paclitaxel in HEY tumor xenografts caused a remarkable antitumor effect. Tumor regression was accompained by a significant inhibition of VEGF,