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Gene expression profiles in rat liver slices after treatment with different hepatic tumour promotors

G. Werle-Schneider[1], K. Schüssler[1], C.K. Behrens[2], M.C. v. Brevern[2], J. Scheel[2], T. Storck[2], D. Mueller[3], R. Gloeckner[3], H. Bartsch[1], A. Bach[2], W. Beerheide[1], M. Bartelmann[1]. *[1]Toxicology and Cancer risk factors, German Cancer Research Center, Heidelberg, Germany; [2]AXARON Bioscience AG, Heidelberg, Germany; [3]Pharmacology and Toxicology, Friedrich-Schiller-University, Jena, Germany*

Promoting agents in the liver can be classified according to their effect on gene expression profiles in rat liver after *in vivo* treatment. For high-throughput drug screening there is a need for *in vitro* assays which should reflect the *in vivo* conditions. Using DNA microarrays containing 1600 genes of the rat genome, the utility of rat liver slices in gene expression studies was investigated. Precision-cut rat liver slices were prepared and exposed to phenobarbital (PB), alpha-hexachlorocyclohexane (HCH), dehydroepiandrosterone (DHEA) and ethinylestradiol (EE) for 24h. For comparison, livers of animals treated *in vivo* with these agents were analysed. The responses *in vitro* observed in drug metabolising genes, including cytochrome P-450 genes (CYPs), epoxide hydrolases, UDP-glucuronosyl transferases (UGTs), glutathione transferases (GSTs) and peroxisomal genes corresponded well with published data. Expression of UGTs, GSTs and several CYP genes, like members of the CYP2 and CYP3 families were increased in rat liver slices treated with enzyme inducers like PB and HCH. After treatment with the peroxisome proliferator DHEA, the expressed genes were those predominantly involved in lipid metabolism and β-oxidation; genes of the CYP4A subfamily were also upregulated. After exposure of rat liver slices to EE, the expression of only a few genes, like steroid 3-alpha-dehydrogenase, which is involved in the steroid metabolism and the Bcl-2 apoptosis related gene was changed. To establish gene expression profiles, a two-dimensional hierarchical clustering analysis was performed. The clusters for PB and HCH, either generated in the liver of treated rats and in exposed rat liver slices were similar. The expression profiles of DHEA-treated rat liver slices and those obtained in rat liver after *in vivo* treatment with DHEA or the peroxisome proliferator WY-14,643 were similar. However the expression profiles observed in liver after *in vitro* and *in vivo* exposure by EE was distinctly different from those produced by the other chemicals. In conclusion we could demonstrate that gene expression profiles for compounds that act via similar mechanisms show common effects on transcription *in vivo* and in rat liver slices. Our results support the use of rat liver slices as tool in predictive toxicology, i.e. for the development and screening of new drugs. Supported in part by the Bio-Regio-Project 0311942

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PX-478, a potent inhibitor of hypoxia-inducible factor-1 (HIF-1) and antitumor agent

S.J. Welsh[1], R.R. Williams[1], D.L. Kirkpatrick[2], G. Powis[1]. *[1]Arizona Cancer Center, Powis Labs, Tucson; [2]ProlX Pharmaceuticals, Tucson, Arizona, USA*

Hypoxic cancer cells are found in all solid tumors and are the most difficult cancer cells to kill. HIF-1 is a transcriptional activator that plays a critical role in mediating the growing tumors response to hypoxia. Downstream genes activated by HIF-1 lead to increased glycolysis, resistance to apoptosis and increased tumor angiogenesis. HIF-1 is a heterodimer consisting of a HIF-1alpha subunit whose rapid breakdown under aerobic conditions is mediated by the Von Hippel Lindau protein (pVHL), and an oxygen-insensitive HIF-1β (ARNT) subunit. HIF-1alpha protein is elevated many fold in cells under conditions of hypoxia (<1-5% O2) and has been found to be increased in many human tumors but absent in normal tissue. HIF-1alpha is, thus, an attractive target for cancer drug discovery with the potential to decrease cancer cell survival and inhibit angiogenesis. We have identified PX-478 as a novel small molecule inhibitor of HIF-1alpha (IC_{50} of 5 and 7 μM in MCF-7 human breast and HT-29 human colon cancer cells, respectively). PX-478 inhibits HIF transactivating activity in the same cells (IC_{50} 7-9 μM) possibly by inhibiting thioredoxin reductase which is required for the oxygen dependent degradation of HIF-1alpha. PX-478 also inhibits hypoxia-induced vascular endothelial growth factor formation by MCF-7 and HT-29 colon cancer cells (IC_{50} 7-9 μM). PX-478 shows a selective growth inhibition of RCC4/pVHL- human renal cancer cells which have constitutively elevated HIF-1 alpha, compared to RCC4/pVHL+ cells. PX-478 administered as a single course, ip daily at 60 to 120 mg/kg for 4 days, to scid mice with established (0.2 to 0.5 g) human tumor xenografts gave tumor regressions and excellent dose-dependent tumor growth inhibition of MCF-7 breast cancer (T/C 92%, growth delay 42 days), HT-29 colon cancer (T/C 85%, growth delay 27 days) and PC3 prostate cancer (T/C 95%, growth delay >35 days). Peak plasma levels of PX-478 after an ip dose of 150 mg/kg were 550 μg/ml and t1/2 alpha 23 min and t1/2β 93 min. Immunohistochemical measurement showed almost complete depletion of HIF-1 alpha in MCF-7 tumor xenografts in scid mice administered PX-478 60 mg/kg ip for 4 days. Thus, PX-478 is the first of a novel class of HIF-1 inhibitors that causes depletion of HIF-1 alpha in tumors and inhibition of HIF activity. PX-478 has excellent activity against established human tumor xenografts causing tumor regressions and prolonged growth delay after only a single course of treatment.

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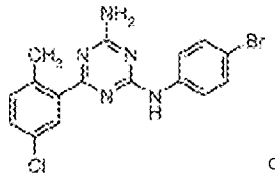
Inhibition of lysophosphatidic acid acyltransferase-beta (LPAAT-b) by CT-32228 inhibits activation of RAS-RAF-Erk and PI3K/AKT/m-TOR pathways and selectively induces tumor cell apoptosis

M. Coon[1], A. Ball[1], L. Bonham[1], J. Pound[1], S. Ap[1], P. de Vries[1], J.W. Singer[1]. *[1]Cell Therapeutics, Inc., Seattle, USA*

Lysophosphatidic acid acyltransferase-beta (LPAAT-b), an intrinsic membrane protein that catalyzes the synthesis of phosphatidic acid (PA) from lysoPA, has been implicated in cellular transformation. Elevated cellular PA has been documented in models of ras-transformation and it has been shown to affect tyrosine kinase signaling cascades, vesicle trafficking and cell motility through direct interactions with specific proteins or in a more generalized manner by altering locally the physical properties of membrane bilayers. Previously we reported that LPAAT-b cooperated with RAS or RAF in the activation of ERK1/2 and enhanced germinal vesicle breakdown in Xenopus oocytes. We show here that overexpression of LPAAT-b in NIH/3T3 cells is associated with proliferation in low serum and tumorigenicity in nude mice. Removal of the overexpressed gene reversed these parameters of transformation. RNAi knockdown of LPAAT-b in DU145 prostate cancer cells was potently antiproliferative and induced apoptosis in IM-9 myeloma cells. Isoform-specific nanomolar inhibitors of LPAAT-b activity typified by CT-32228 [N-(4-bromophenyl)-6-(5-chloro-2-methylphenyl)-[1,3,5]triazine-2,4-diamine] blocked translocation of RAF and activation of Erk and PI3K/Akt pathways in normal activated endothelial and vascular smooth muscle cells (VSMC).



CT-32228

Treatment of Angiotensin II or VEGF treated VSMC or human microvessel endothelial cells with CT-32228 induces profound, dose dependent decreases in the phosphorylation states of effectors of both pathways including Erk1/2, Akt, mTOR, GSK3beta, p70S6k and p90RSK. Both of these proliferative and cell survival signaling cascades involve PA-regulated processes, which could explain the anti-LPAAT-b effects. LPAAT-b inhibitors are potently antiproliferative and cytotoxic in all tumor cell lines tested and induce Type I and Type II apoptosis pathways as shown by the expression and activation of TRAIL/Apo-2L receptosome complexes and subsequent activation of Caspase 9 and Bax. In contrast, normal human bone marrow progenitors and activated naive murine T cells are resistant to both the antiproliferative and apoptosis effects of CT-32228. These data indicate that LPAAT-b plays an important role in tumor cell survival and that LPAAT-b represents a novel therapeutic target for cancer treatment.

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G-quadruplex interaction of 3,6,9-trisubstituted acridines leads to specific telomerase inhibition, induction of senescence and telomere shortening

F. Dai[1], J. Perez[2], A.P. Reszka[2], G. Chessari[2], M.A. Read[2], A.M. Burger[1], S. Neidle[2]. *[1]Tumor Biology Center at the University of Freiburg, Freiburg i.Br., Germany; [2]The School of Pharmacy, University of London, Cancer Research UK Biomolecular Structure Unit, London, UK*

We have previously shown that acridines with appropriate aminoalkylamido disubstitution can bind to G-quadruplexes formed from human telomeric DNA sequences, and that this interaction also inhibits telomerase en-