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MKP-1/CL100 activity modulates cisplatin responses in non-small cell lung carcinoma

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Non-small cell lung carcinoma (NSCLC) represents the most abundant and therapy refractory subclass of lung cancer. Improving apoptosis induction in NSCLC represents a logical way to advance in treatment is this tumor. In NSCLC derived cell lines H-460 and H-1299, with different degrees of sensitivity to cisplatin, this drug differentially induces activation of N-terminal c-Jun Kinase, that in turn mediates induction of apoptosis. MKP-1/CL100 a negative regulator of JNK, is up-regulated in H-460 cells. By expressing a SiRNA vector for MKP-1 we found that in H-460 MKP-1/SiRNA expressing cells, cisplatin induces more efficiently activation of JNK and p38 and that correlated with an increase in 10-fold sensitivity to cisplatin. By contrast to H-460, no differences in survival were observed in H-1299 expressing the MKP-1SiRNA. Moreover, expression of a SiRNA to MKP-2, a CL100 related phosphatase, showed no effect in H-460 cell viability to cisplatin treatment. MKP-1SiRNA/H-460 cells grow both slower in nu/nu mice and also show more susceptibility to cisplatin than parental cells, hence resulting in an impaired growth of the tumor in mice. When analyzing surgical samples of NSCLC patients we have found that expression of MKP-1 showed a strong nuclear staining for tumor cells while in normal bronchial epithelia, MKP-1 was localized both in cytoplasm and nuclei. Altogether, the results showed that inhibition of MKP-1 expression contributes to a slower growth of cell in mice and also to an increase of cisplatin induced cell death in NSCLC, suggesting that MKP-1 constitutes an attractive target for sensitizing cells to cisplatin in human tumors.

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ZD4054 specifically inhibits endothelin A receptor-mediated anti-apoptotic effects, but not endothelin B receptor-mediated pro-apoptotic effects

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The clinical use of endothelin antagonists in oncology is a new and interesting area of study. In most oncology settings, blockade of only one of the two endothelin receptor subtypes (endothelin A receptor [ET_A]) infers benefit, while blockade of the other (endothelin B receptor [ET_B]) may lead to undesirable effects. Evidence suggests that stimulation of the ET_B receptor increases apoptosis, therefore antagonism of the ET_B receptor could inhibit apoptosis of tumour cells. We and others have shown that ET_B receptor expression is reduced, but still present, in clinical samples from a number of solid tumours (but not in melanoma and glioma where the ET_A and ET_B receptor seem to have opposite roles); a finding which is consistent with its pro-apoptotic role. We have also shown previously that ZD4054 is a specific ET_A receptor antagonist because it binds to the ET_A receptor with high affinity *in vitro,* but has no measurable affinity for the ET_B receptor (Curwen and Wilson. Eur J Cancer 2002;38[Suppl 7]: S102). Here we demonstrate that ZD4054 inhibits ET_A-mediated anti-apoptotic events while allowing pro-apoptotic signalling via ET_B in both human and rat epithelial cell lines *in vitro*.

We confirm the findings of Wu-Wong et al (Biochem J 1997;328:733–7) showing that endothelin 1 (ET-1) inhibits apoptosis induced by serum starvation and show that a specific ET_A receptor antagonist BQ123 inhibits this response. We also demonstrate that ZD4054 inhibits this ET_A-mediated process in the rat A10 and the human VLTR 16 smooth muscle cell lines in a dose-responsive manner. In contrast, selective activation of the ET_B receptor by the peptide agonist BQ3020 induced pro-apoptotic signalling in the same lines, a response which was not reversed by ZD4054.

The data presented here show that ZD4054, a specific ET_A receptor antagonist, blocks ET_A receptor-mediated anti-apoptotic signals leading to apoptosis, while at the same time, allows pro-apoptotic signalling to continue via the ET_B receptor. The specificity of ZD4054, together with the opposing roles of ET_A and ET_B receptors in mediating apoptosis, suggests that ZD4054 has the potential to block the pathological processes mediated by the ET_A receptor, but allow the beneficial processes mediated by the ET_B receptor to proceed.

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Characterization of novel therapeutic receptor target candidates for treatment of small cell lung cancer

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An oligonucleotide microarray analysis on a panel of 21 small cell lung cancer (SCLC) cell lines and 8 xenografts, as well as data from 5 resected tumors (Bhattacharjee A, et al. (2001) Proc. Natl. Acad. Sci. USA **98**:13790) revealed expression of a large number of highly expressed genes, including genes encoding cell surface receptors. Aiming to identify novel receptor targets for treatment of SCLC, some of these receptors were selected for assessment of therapeutic potential. Receptor selection criteria included high mRNA expression in SCLC (cell lines, xenografts and tumors) and low or absent expression in all or most adult normal human tissues. The analysis resulted in identification of approximately 120 genes of interest. From this selection a small number of genes were chosen for further studies on the basis of availability of identified ligands.

Selected receptors include the ionotropic Glutamate Receptor Subunit 2 (GRIA2), the metabotropic Glutamate Receptor 8 (GRM8) and the Neuronal Pentraxin Receptor (NPTXR). GRIA2, GRM8 and NPTXR mRNA expression has been confirmed by semi-quantitative RT-PCR and protein expression was verified by western blot analysis of total cell lysates from all the cell lines and xenografts used for the microarray analysis. Western blotting of membrane fractions from a selection of SCLC cell lines confirmed the localization of the three receptors to the cell membrane.

For GRIA2 no specific binding could be detected using the radio ligand ^{3}H-AMPA. However preliminary results showed a concentration dependent antiproliferative response for SCLC cell lines expressing GRIA2 upon exposure to the ionotropic glutamate receptor antagonist GYKI-52466.

A recombinantly produced ligand of the NPTXR was found to associate with the cell surface in a NPTXR expressing SCLC cell line, but not to a fibroblast cell line without expression of NPTXR.

These results indicate that the ligand binding functionality of GRIA2 and NPTXR is retained in SCLC and these receptors may therefore candidate as novel targets for treatment of SCLC.

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Membrane gamma-glutamyltransferase as a target for intervention on several redox-modulated functions of the cancer cell

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Background: Biomolecules involved in signal transduction and gene expression are sensitive to prooxidants, and a "redox regulation" has been described for many of them. Prooxidants originate from several sources; our previous work identified membrane gamma-glutamyltransferase (GGT) as one such source (Biochem. Pharm. 64, 1029, 2002). ROS and other free radicals are in fact produced during GGT-mediated metabolism of glutathione (GSH).

Methods: Several tumor cell lines are being used. Clones transfected for increased GGT expression have been obtained. Cells are studied in conditions of GGT stimulation vs. GGT inhibition. Determinations are aimed to investigate the effects of GGT activity on: i) cellular low mol. wt. thiols (HPLC studies); ii) the cellular balance of critical redox-active compounds, e.g. vitamin C (HPLC); iii) thiol redox status of selected membrane proteins (immunoblot, Elisa and FACS studies); iv) ligand binding affinity of membrane receptors (125I-labelled agonists); v) selected cell functions (proliferation; viability and apoptosis; adhesion); vi) resistance of cells to platinum-based drugs.

Results: i) Cysteinyl-glycine (CG) produced by GGT forms mixed disulfide bonds with cellular protein; ii) extracellular ascorbic acid is easily oxidized by GGT to dehydroascorbate, i.e. a form of the vitamin that is promptly absorbed; thus, GGT expression facilitates the supply of vitamin C; iii) at least five distinct redox forms of the TNFR1 receptor were identified, whose relative abundance is dependent on the GGT activation status; iv) TNFR1 redox status is reflected in changes of its ligand binding affinity for TNF-alpha; v) redox activity of GGT affects the basal levels of poly(ADP-ribose) polymerase activity; vi) GGT expression increases cell resistance to cisplatin toxicity, likely due to formation of complexes with CG outside cells.

Conclusions: Prooxidant reactions initiated at the cell surface during GGT-mediated catabolism can have profound effects on a series of cellular parameters connected with viability, apoptosis and drug resistance. As expression of GGT is frequent in human neoplasms, and often