ZIO-101 is clinically active. MTD, 500 mg/me2/d, is >50-fold higher than the dose of As_2O_3. ZIO-101 is a promising drug for further development: phase-2 trials are starting.

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Bcl-2 nineteen kilodalton interacting protein (BNIP3) is a transcriptional regulator in glioma cells that acts as a survival factor, silencing the expression of pro-apoptotic genes

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Background: Novel mechanisms have recently been proposed for the Bcl-2 family members Bok, Bcl-2 and BID in the nucleus. They have been found to play a role in induction of apoptosis, alteration of gene transcription, and the DNA damage response. We have found that BNIP3 (a BH3-only member of the Bcl-2 family) is expressed in the nucleus of astrocytic cells under normal conditions, and in a subset of glioblastoma multiforme tumors (GBMs). We present that BNIP3 plays a novel role in the nucleus of glial cells by binding to the promoter/silencer regions of genes involved in induction of cell death or apoptosis, and silences these genes. If low expression of these genes results in a survival advantage, this may explain why expression of nuclear BNIP3 is selected for in GBMs.
Materials and Methods: Formaldehyde and cisplatin crosslinking of proteins to DNA was completed and: 1) protein was extracted and analyzed by western, 2) DNA was extracted by chromatin immunoprecipitation (ChIP) with the BNIP3 antibody. A gel shift assay was completed with probes specific for genes identified in the ChIP. Proteins isolated from a His-tag pull down with a His-BNIP3 construct were separated by 2-d gel electorophoresis. Spots were picked, sent for mass spectrometric analysis and confirmed by co-immunoprecipitation (co-IP). Stable transfection of nls-BNIP3 and shRNA-BNIP3 constructs were completed in U251 cells and these cells were treated with temozolomide (TMZ) and hypoxia.
Results: We have determined that the over-expression of BNIP3 in the nucleus in glioma cells provides a survival advantage against hypoxic stress as well as TMZ treatment. BNIP3 binds to a consensus sequence in the promoter/enhancer regions of genes involved in apoptosis and cell death. One of these genes is the PDCD8 gene, which codes for the AIF (apoptosis inducing factor) protein. We have confirmed in U251 cells that overexpression of BNIP3 in the nucleus decreases the level of protein expression of AIF, and concurrently stable expression siRNA for BNIP3 leads to an increase in AIF expression. Also, we have identified a subset of DNA/RNA binding proteins that interact with BNIP3 in the nucleus of glioma cells. PSF (polypyrimidine tract associated splicing factor) has been confirmed to interact with BNIP3 by co-IP.
Conclusions: We have found that nuclear BNIP3 downregulates AIF expression in astrocytes leading to resistance to TMZ and hypoxia-induced cell death. The interaction of BNIP3 with PSF indicates that BNIP3 may also regulate specific genes by alternative splicing. Nuclear BNIP3 therefore would be selected for in GBM tumors because it would provide a survival advantage in hypoxic conditions created in the interior of the tumor.

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Hyaluronan induces apoptosis through CD44 in activated T lymphoma cells

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Altered expression of the cell adhesion molecule CD44 is associated with metastasis in several human cancers and numerous studies have implicated the binding of CD44 to its primary ligand hyaluronan (HA) as being responsible. The CD44-HA interaction may also be important in regulating cell survival as binding to HA promotes anchorage-independent cell growth and mediates resistance to drug-induced apoptosis in human lung carcinoma cells. In contrast, anti-CD44 antibodies can inhibit proliferation and induce apoptosis in human leukemia cells, while in mouse T lymphoma cells, HA both enhances and protects from apoptosis depending on the type of drug used. Together, these findings suggest that the effect of CD44 on apoptosis may be cell type and condition specific. To better understand the role of the CD44-HA interaction in the induction of apoptosis in T cells, human Jurkat T lymphoma cells were transfected with CD44 or CD44 containing mutations that either increase or prevent binding to HA. Jurkat cells were stained with Annexin V-FITC and propidium iodide and analyzed by flow cytometry to measure apoptosis. Cells were found to be equally sensitive to apoptosis induced by treatment with staurosporine or an anti-CD95 antibody, suggesting that CD44 expression alone did not affect apoptosis. However, the activation of CD44 transfected Jurkat cells with immobilized anti-T cell receptor (TCR) antibody or phorbol myristate acetate (PMA) increased binding to HA and resulted in apoptosis in the presence of HA. Apoptosis was enhanced during activation in cells expressing high HA-binding mutant CD44, while it did not occur in cells transfected with mutant CD44 incapable of binding HA. Similarly, incubation with an HA blocking anti-CD44 antibody or hyaluronidase prior to activation prevented apoptosis. While it has been previously shown that CD44-deficient mouse T cells are resistant to activation-induced cell death, our data are the first to demonstrate that binding of CD44 to HA can induce apoptosis in activated T cells.

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c-FLIP regulates the interaction between interferon-gamma and doxorubicin in breast cancer cells

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Combination treatment regimens that include the topoisomerase-II (topo-II)-targeted drugs, such as doxorubicin, are widely used in the treatment of both early and metastatic breast cancers. Previously we demonstrated that combinations of these drugs with IFN-γ potentiated apoptosis in breast cancer cells in a STAT-1-dependent manner. In this study we found that this synergy was caspase-8-dependent. Furthermore, we found the enhanced apoptosis was mediated by the death receptors Fas and DR5. However, the cognate ligands of these receptors were not constitutively expressed or up-regulated by either IFN-γ or doxorubicin in these cells, suggesting that a ligand-independent signalling mechanism was stimulating the activation of these receptors. In addition, we found that IFN-γ dramatically down-regulated the expression of the caspase-8 inhibitor, cellular-FLICE-like inhibitory protein (c-FLIP), in MDA-435 cells, in a STAT1 and IRF-1-dependent manner. Characterisation of the functional significance of c-FLIP modulation by siRNA gene silencing and stable over-expression studies, revealed it to be a key regulator of IFN-γ and doxorubicin-induced apoptosis in MDA-435 cells. Analysis of a wider panel of breast cancer cell lines also indicated that c-FLIP was a key regulator of IFN-γ/doxorubicin-induced cell death. Furthermore, *c-FLIP* gene silencing also sensitised MDA-435 cells to the other topo-II inhibitors, etoposide and mitoxantrone, as well as the topo-I inhibitor, SN-38. These results indicate that c-FLIP plays a pivotal role in the modulation of drug-induced apoptosis in breast cancer cells and may have important clinical applications as a therapeutic target and/or a marker of chemosensitivity in tumour cells.

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Kinetic modelling of R8BH3BID induced BAX/BAK activation dynamics in Non-Small Cell Lung Cancer cells

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Non-small cell lung cancer (NSCLC) exhibits de novo resistance to chemotherapy. Suppression of apoptosis, a hallmark of NSCLC may contribute significantly to the chemoresistant phenotype. Activation of proapoptotic BCL-2 family proteins BAX and BAK constitutes a critical switch that initiates mitochondrial outer membrane permeabilization (MOMP) and inner membrane permeabilization (MIMP). Regulation of BAX/BAK oligomerization kinetics in NSCLC may impact susceptibility to chemotherapy induced apoptosis, however robust quantitative methods for direct estimation of MOMP/MIMP kinetics have not been explored. R8BH3BID peptide, a direct activator of BAX/BAK conformation change, was synthesized and as an N-terminal D-octoargarginine conjugate (R8), validated by electrospray mass spectroscopy and purified by high performance liquid chromatography. Analogues containing negative control point mutant, hexanoic acid spacing between R8 and BH3BID and N-terminal acetyl capping were equipotent. Rapid cell uptake was verified using carboxyfluorescein conjugated analogue which localized to mitochondria, and alpha-helical secondary structure confirmed by circular dichronism spectroscopy. Exogenous R8BIDBH3 (50microM) mediated rapid BAX conformation change, MOMP (cytochrome C, SMAC release), and MIMP measured by tetramethylrhodamine ester (TMRE) within 3 hours. At single cell resolution, MIMP exhibited stochastic behaviour. A machine vision algorithm developed to detect loss of TMRE fluorescence by live cell microscopy, enabled modelling of the survival function by the product limit estimator. NSCLC cells (H460) co-expressing BCL-2, BCL-XL, MCL-1 and BCL-W by RTPCR and western analysis, exhibited significantly faster R8BIDBH3 induced MIMP kinetics compared with human bronchial epithelial cells (BAES2B) lacking these antiapoptotic proteins, suggesting