reduction of FL-RAGE. However, brefeldin A does not affect the TNF-induced NF-κB activation in these cells, indicating that the drug did not interfere with TNF-signaling in general.
In summary, TNF-induced cell death requires a nucleocytoplasmic translocation of the cell surface receptor RAGE. Furthermore, the cell surface RAGE may be a promising target for the induction of cell death in tumor cells.

476 POSTER

Inhibition of EMMPRIN (CD147) sensitizes human breast cancer cells to anoikis

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Overexpression of extracellular matrix metalloproteinase inducer (EMMPRIN or CD147), a member of the immunoglobulin family and a glycoprotein enriched on the surface of tumor cells, promotes invasion, metastasis, growth and survival of malignant cells, and confers resistance to some chemotherapeutic drugs. However, the molecular mechanisms underlying the actions of EMMPRIN are not fully understood. In this study we sought to determine whether EMMPRIN contributes to the malignant phenotype of breast cancer by inhibiting anoikis, a form of apoptosis induced by loss or alteration of cell-cell or cell-matrix anchorage, and to explore the signaling pathways involved. We found that human breast carcinoma cells expressing high levels of EMMPRIN formed aggregates with large surface area, had higher viability, and were resistant to apoptosis in the absence of attachment. Knockdown of EMMPRIN expression by RNA interference (siRNA or shRNA) sensitized those cancer cells to anoikis, as demonstrated by activation of caspase-3, increased DNA fragmentation and decreased cellular viability. Furthermore, we observed that the accumulation of Bim, a pro-apoptotic BH3-only protein, was reduced in EMMPRIN-expressing cells, and that silencing of EMMPRIN expression elevated Bim protein levels and enhanced cellular sensitivity to anoikis. Inhibition of Bim expression by siRNA decreased the sensitivity to anoikis in cells with low EMMPRIN. Treatment of cells with a MEK inhibitor (U0126) or proteasome inhibitor (epoxomicin) also upregulated Bim accumulation and rendered cells sensitive to anoikis. These results indicate that expression of EMMPRIN protects cancer cells from anoikis, and this effect is mediated by a MAP kinase-dependent reduction of Bim via proteasomal degradation. Since anoikis deficiency is a key feature of neoplastic transformation and invasive growth of epithelial cancer cells, our study on the role of EMMPRIN in anoikis resistance and the mechanism involved underscores the potential of EMMPRIN expression as a prognostic marker and novel target for cancer therapy.

477 POSTER

Inhibition of mTOR or apopotic pathway induces autophagy and radiosensitizes PTEN null prostate cancer cells

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Background: The PI3K/Akt pathway plays a critical role in oncogenesis, and dysregulation of this pathway through loss of PTEN suppression is a particularly common phenomenon in aggressive prostate cancers. The mammalian target of rapamycin (mTOR) is a downstream signaling kinase in this pathway, exerting prosurvival influence on cells through the activation of factors involved in protein synthesis. The mTOR inhibitor rapamycin and its derivatives are cytotoxic to a number of cell lines; recently, mTOR inhibition has also been shown to radiosensitize endothelial and breast cancer cells in vitro.
Hypothesis: Because radiation is an important modality in the treatment of prostate cancer, we tested the ability of the mTOR inhibitor RAD001 (everolimus) to enhance the cytotoxic effects of radiation on two prostate cancer cell lines, PC-3 and DU145.
Results: We found that both cell lines became more vulnerable to irradiation after treatment with RAD001, with the PTEN deficient PC-3 cell line showing the greater sensitivity. This increased susceptibility to radiation is primarily driven by induction of autophagic cell death. Furthermore, we demonstrate that blocking apoptosis with caspase inhibition and Bax/Bak siRNA in these cell lines enhances radiation-induced mortality in an autophagic dependent process.
Conclusion: Together, these data highlight the emerging importance of mTOR as a molecular target for therapeutic intervention, and lend support to the idea that non-apoptotic modes of cell death may play a crucial role in improving tumor cell kill.

478 POSTER

Gefitinib reverses TRAIL resistance in human bladder cancer cell lines via inhibition of AKT-mediated XIAP expression

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Background: Inhibitors of the epidermal growth factor receptor (EGFR) display activity in subsets of solid tumors, but identifying responsive tumors prospectively has been elusive, and it is not clear how to best exploit the biological effects of EGFR inhibitors.
Materials and Methods: We measured DNA synthesis by 3H-thymidine incorporation and DNA fragmentation associated with apoptosis by propidium iodide staining and FACS analysis. We quantified the expression of various target proteins by immunoblotting. We knocked down expression of AKT or XIAP by transient transfection with commercially available siRNA constructs. We studied the effects of therapy on the growth of orthotopic 253J B-V xenografts in nude mice.
Results: The EGFR inhibitor gefitinib (ZD1839, Iressa) blocked cell proliferation at relevant concentrations in 7/18 human bladder cancer cell lines. Sensitivity to gefitinib was loosely associated with expression of E-cadherin and lack of expression of vimentin characteristic of tumor cells that have not undergone the epithelial-to-mesenchymal transition (EMT). The drug had modest effects on DNA fragmentation and also failed to promote apoptosis induced by conventional chemotherapeutic agents (gemcitabine and paclitaxel). However, it did interact with recombinant human tumor necrosis factor related apoptosis-inducing ligand (TRAIL) to induce high levels of apoptosis in gefitinib-sensitive but not gefitinib–resistant lines. The molecular mechanisms involved downregulation of active AKT and XIAP expression and were mimicked by chemical inhibitors of the PI3 kinase/AKT but not of the MEK/ERK pathway. Furthermore, direct siRNA-mediated knockdown of AKT resulted in downregulation of XIAP and TRAIL sensitization, and knockdown of XIAP itself was sufficient to reverse TRAIL resistance. The effects of gefitinib plus TRAIL on the growth of TRAIL-resistant orthotopic 253J B-V xenografts will be presented.
Conclusions: Our results demonstrate that EGFR pathway activation limits TRAIL-induced apoptosis via an AKT- and XIAP-dependent mechanism in EGFR-dependent human bladder cancer cells. The data provide the conceptual framework for a further evaluation of the combination in relevant preclinical models and clinical trials in patients.

479 POSTER

ZIO-101: a new organic arsenic in advanced cancers

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Background: Arsenics are potent anti-cancer drugs. Organic arsenics are much less toxic than inorganic arsenics (like arsenic trioxide [As_2O_3]). ZIO-101 (S-dimethylarsino-glutathione; Figure), a new organic arsenic, is active against diverse cancers in experimental models and has a LD50 about 50-fold higher than As_2O_3. ZIO-101 is 5–10-fold more efficient in entering cancer cells than As_2O_3. Finally, ZIO-101 more specifically affects the pro-apoptotic signaling pathway than does As_2O_3. These features result in more damage to mitochondria and more cell-killing with ZIO-101 than with As_2O_3.
Methods: Combined data from 3 phase-1 ongoing studies evaluating safety, activity and pharmacokinetics of ZIO-101 in subjects with advanced cancers failing many prior therapies. Starting dose was 78 mg/me2/d IV for 5 d every mo with 20−40% dose increases.
Results: 49 subjects were treated including 29 with diverse advanced solid cancers and 20 with blood and bone marrow cancers. Detailed data are available on 33; data in 16 more will be presented. Median age is 61 y (43−85 y); 16 were male. The maximum administered dose (MAD) was 595 mg/me2/d, the estimated maximum tolerated dose (MTD), 500 mg/me2/d and the dose limiting toxicity (DLT), transient confusion and ataxia. Clinical benefit was reported in 10 subjects (30%) including acute myelogenous leukemia (AML) and solid cancers (colorectal, kidney, head and neck and pancreas cancers). 3 subjects with AML had substantial deceases in blood leukemia cells, and 1 subject had a reduced RBC transfusion need. 5 subjects with solid cancers had stable disease for 3+ to 7+ mo and 1 subject had a mixed response. Therapy with ZIO-101 at the MTD was safe: fatigue was the only toxicity $\geqslant$ grade-2 occurring in $\geqslant$ 25% of subjects. Clinically-important QTc-prolongation, a limitation of As_2O_3, did not occur. Pharmacokinetic (PK) studies at 214 mg/me2/d: tmax = 1 h (no SD), Cmax = 685 μg/L (SD ±130 μg/mL), t1/2 = 13.9 h (SD ±0.3 h) and AUC0-∞ = 14.9 mg h/L (SD ±2.6 mg h/L).
Conclusions: Clinical and PK data show ZIO-101 is safe at doses resulting in blood levels with substantial anti-cancer activity in experimental models.

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.

480 POSTER

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.

481 POSTER

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.

482 POSTER

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.

483 POSTER

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