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.

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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.

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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.

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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.