stimulation of downstream effectors ROCK, LIMK2 and ADF/destrin. Furthermore, dominant negative Rho/Cdc42 or pharmacological inhibitors of ROCK inhibited both actin organization and apoptosis in DU145 cells. Interestingly, RhoA/B and ROCK were also implicated in mAR-dependent actin polymerization and apoptosis in LNCaP cells, acting most probably downstream of FAK/PI-3K/Rac signaling.
Conclusions: Rho GTPases are major mAR effectors controlling actin reorganization and apoptosis in prostate cancer cells.

263 POSTER

IGF-1 receptor stimulation overrides microenvironment-derived tumour cell quiescence

A.H. Kyle[1], J.H.E. Baker[1], F. Moosvi[1], A.I. Minchinton[1]. *[1]BC Cancer Research Centre, Medical Biophysics, Vancouver, Canada*

Purpose: To study the regulation of cellular proliferation, quiescence and necrosis in the context of the tumour microenvironment.
Background: Most conventional chemotherapy-based therapies rely on the elevated proliferation status of cancer cells as a means of achieving a therapeutic index. However, the microenvironment in solid tumours often limits the efficacy of anti-proliferative therapies by generating a quiescent tumour cell subpopulation. This study investigates the factors driving tumour microenvironment derived cellular quiescence, employing tumour mapping techniques and a 3-D cell culture model, multilayered cell culture (MCC), in which tumour cells are grown into discs of tissue.
Methods: *Tissue mapping studies*: Solid tumours and MCC-discs were grown using HCT-116 and HT29 tumour cells under a range of conditions and then sectioned and immunostained to examine the variation of proliferation, apoptosis and hypoxia with depth into tissue. *Nutrient flux studies*: MCC-discs were used to separate two reservoirs of a flux apparatus and the tissue penetration of a panel of 15 amino acids was evaluated.
Results: Proliferating cells in HCT-116 tumour xenografts cluster around micro-vessels and are significantly reduced at a distance of 50 μm away from vessels while the hypoxia marker pimonidazole reaches maximal intensity at a distance of 100–150 μm. The flux of a panel of 15 amino acids through MCC-discs indicated a similar flux of all amino acids with the exception of glutamine, which passed through at 20% the rate of the others. However, supplementation of glutamine did not alter the proliferation profile in MCC-discs. 95% oxygen induced uniform proliferation throughout MCCs, indicating that under elevated oxygenation the supply of nutrients and glucose and the removal of waste products weren't limiting the depth of proliferation. Stimulation of the IGF-1 receptor was able to more than double the depth of proliferation in MCC-discs, pushing proliferation out to the border of hypoxia.
Conclusions: At intermediate distances into tissue (0–100 μm) the supply and removal of nutrients and waste products were not rate limiting factors in the determination of proliferation status; stimulation of the IGF-1 receptor alone was able to induce proliferation in quiescent cells. At greater distances (100–150 μm) hypoxia, and possibly the subsequent build-up of secreted factors, appeared to be the dominant factors which limited proliferation.

264 POSTER

A novel arsenical, darinaparsin, induces apoptosis in arsenic trioxide-resistant and MRP1/ABCC1-overexpressing cell lines

K.K. Mann[1], Z. Diaz[1], S. Marcoux[1], M. Kourelis[1], M. Colombo[1], P.B. Komarnitsky[2], G. Abbadessa[2], W.H. Miller Jr.[1]. *[1]Segal Cancer Centre at the Lady Davis Institute for Medical Research SMBD Jewish General Hospital McGill University, Oncology, Montreal, Canada; [2]Ziopharm Oncology Inc., Oncology, Boston, USA*

Inorganic arsenic trioxide (As_2O_3) has been proven to be a highly effective treatment for acute promyelocytic leukemia (APL). However, other cancers do not respond well to this form of arsenic at clinically achievable doses. We tested a novel organic arsenical, S-dimethylarsino-glutathione (darinaparsin, ZIO-101), for efficacy in various malignancies *in vitro*. We find that darinaparsin is significantly more potent than As_2O_3 at mediating apoptosis *in vitro* in a variety of malignant cell lines and is highly active against APL cells selected for As_2O_3 resistance. We provide evidence that darinaparsin triggers apoptosis by inducing signaling pathways that do not completely overlap with As_2O_3. Darinaparsin induces apoptosis and oxidative stress to a greater extent than As_2O_3, although, like As_2O_3, darinaparsin-induced toxicity is dependent on JNK activation. However, darinaparsin does not induce PML-RARα degradation or rearrange PML nuclear bodies in the APL NB4 cell line, nor is its toxicity increased by depletion of GSH. Treatment with darinaparsin results in higher intracellular accumulation of arsenic when compared to treatment with As_2O_3. This may be explained by our finding that As_2O_3, but not darinaparsin, is efficiently exported by ABCC1. These results suggest darinaparsin might have greater therapeutic efficacy than As_2O_3 in tumors that overexpress ABCC1. Overall our studies indicate that darinaparsin efficiently kills tumor cells with increased antioxidant capacity and drug exporters and suggest that darinaparsin may have a broader therapeutic spectrum than As_2O_3. To test this hypothesis, we have initiated a Phase I clinical trial of oral darinaparsin. Early biomarker and safety data will be discussed.

265 POSTER

A MEK1/2 inhibitor, AZD6244 (ARRY-142886), shows beneficial effects when combined with standards of care or novel therapies – mechanistic characterisation suggests a role for apoptosis

S.V. Holt[1], A. Logie[2], B.R. Davies[2], M. Cockerhill[2], N. Haupt[2], N.J. Curtis[2], D.M. Hickinson[2], J.S. McKay[2], P.D. Smith[2], R.W. Wilkinson[2]. *[1]Astrazeneca/Paterson Institute for Cancer Research, Cancer and Infection, Macclesfield, United Kingdom; [2]Astrazeneca, Cancer and Infection, Macclesfield, United Kingdom*

The Ras/Raf/MEK/ERK signalling cascade is used by growth factors to transmit signals from their receptors which regulate gene expression and survival. In a number of human cancers members of this pathway, in particular Ras and B-Raf, are observed to be mutated or aberrantly expressed. Furthermore, it has been demonstrated that abnormal activation of this pathway plays a role in chemotherapeutic drug resistance. Thus, members of this pathway present themselves as attractive anti-cancer drug targets. AZD6244, (ARRY-142886), is a novel, selective ATP uncompetitive inhibitor of MEK1/2 and is currently in phase II clinical trials. In order to support the clinical progression of AZD6244 we have used in vivo xenograft models from mKRAS human tumour cell lines (HCT-116, CaLu-6, SW620) to investigate the potential benefit of combination therapies. AZD6244 in concurrent combination with either docetaxel, irrinotecan, gemcitabine or temozolomide, was shown to have enhanced anti-tumour efficacy compared to single agent treatments. In addition, AZD6244 was analysed in combination with novel, molecularly targeted, agents (e.g. gefitinib) and shown to be efficacious. All the above AZD6244 combinations appear to be well tolerated. In order to determine if dose sequencing could enhance this anti-tumour effect, we looked at a variety of dosing regimes of AZD6244 in combination with docetaxel in HCT116 xenografts. We demonstrated that docetaxel followed by AZD6244 was more efficacious than AZD6244 dosed prior to docetaxel. Ex-vivo studies were undertaken to investigate the contribution of the apoptotic pathway/s. It has been previously shown that activation of the ERK pathway results in the phosphorylation of the pro-apoptotic BH3 only protein Bim (Weston et al, 2003). In AZD6244 treated xenograft models we have demonstrated that preventing ERK phosphorylation results in a 3.5 fold increase in Bim levels compared to controls. Furthermore, the downstream apoptotic markers, cleaved caspase 3 and cleaved PARP, were also upregulated after exposure to AZD6244. It can be rationalised that AZD6244 in combination with other agents may stimulate the apoptosis pathway by upregulating other pro-apoptotic proteins as well as Bim. Therefore, we are currently extending our mechanistic analysis in our combination studies to determine if the anti-tumour responses we have observed are a result of an upregulation in apoptosis.

References

Weston et al., 2003. Oncogene 22: 1281–1293.

266 POSTER

Apoptosis induction in acute myeloid leukemia by inhibition of MEK and MDM2 is strongly associated with the BH3-only proteins Puma and Bim

W. Zhang[1], M. Konopleva[2], J. Burks[1], K. Dywer[1], W. Schober[1], J. Yang[3], T. McQueen[1], M. Hung[4], M. Andreeff[2]. *[1]The University of Texas MD Anderson Cancer Center, Stem Cell Transplantation & Cellular Therapy, Houston, TX, USA; [2]The University of Texas MD Anderson Cancer Center, Stem Cell Transplantation & Cellular Therapy and Leukemia Department, Houston, TX, USA; [3]The University of Texas Graduate School of Biomedical Sciences at Houston and MD Anderson Cancer Center, Molecular and Cellular Oncology, Houston, TX, USA; [4]The University of Texas MD Anderson Cancer Center, Molecular and Cellular Oncology, Houston, TX, USA*

Background: Constitutive activation of the Ras/Raf/MEK/ERK signaling pathway contributes to a series of molecular events that have been observed in more than 50% of primary acute myeloid leukemias (AML) samples and is an independent prognostic factor for the survival of patients with AML. MDM2 (Murine double minute) overexpression inactivates p53

and is present in ⩾50% of AML. These findings provide the rationale for the combined targeting of these pathways.
Material and Methods: The anti-leukemic activity of AZD6244 (ARRY-142886), a small-molecule MEK1/2 inhibitor, and potential synergistic effects with the MDM2 antagonist Nutlin3a were investigated in human AML cell lines with constitutive ERK activation (e.g. OCI-AML3) and in those with lower basal levels of phospho-ERK (U937), as well as in primary AML samples. Effects of the agents on cell growth, apoptosis and signaling were investigated by flow cytometry, immunobloting, immunostatining and real-time PCR analysis.
Results: OCI-AML3, HL60, and MOLM13 cells were more sensitive to AZD6244-induced growth inhibition than U937 cells: the mean IC_{50} values were 0.03, 0.6, and 0.7 μM, respectively, compared with 40.4 μM. Mechanistically, AZD6244 arrested AML cells in G_1 phase by suppressing phosphorylation of ERK and Rb, resulting in modulation of cell cycle-related proteins. AZD6244 was highly active in AML cells with constitutive activation of ERK. However, no significant effects on apoptosis induction were observed at sub-micromolar concentrations. Simultaneous blockade of MEK and MDM2 pathways by combining AZD6244 with the MDM2 antagonist Nutlin3a showed highly synergistic effect in AML cells ($CI_{OCI-AML3}$ = 0.09±0.02 and CI_{MOLM13} = 0.43±0.03) and primary AML samples (CI = 0.65±0.05). Pronounced upregulation of Puma and Bim proteins was observed in combination treatment, which was, at least in part mediated by transcriptional activation by FOXO3a. The latter was upregulated by the suppressions of ERK-mediated phosphorylation of FOXO3a and by MDM2-mediated ubiquitination and degradation. Knockdown of Puma protein resulted in partial rescue of the cells from treatment-mediated apoptosis, while knockdown of Bim protein was less protective. In addition, changes in Mcl-1, Bax, p27 and MDM2 were implicated in apoptosis induced by dual MEK/MDM2 targeting.
Conclusions: AZD6244 has profound cytostatic effects on AML cells with constitutive activation of MEK/ERK signaling. This cytotoxic effect is synergistically enhanced by simultaneous MDM2/p53 targeting with Nutlin3a. Mechanistically, Puma and Bim were identified as critical mediators of apoptosis induced by MEK and MDM2 targeting, which was partially mediated by transcriptional activation of FOXO3a. Results suggest that combinational targeting of MEK and MDM2 with AZD6244 and Nutlin3a has potential as a novel mechanism-based therapeutic strategy for AML.

267 POSTER

Small molecule XIAP inhibitors enhance TRAIL- or anticancer drug-induced apoptosis in childhood acute leukemia cells and overcome Bcl-2-mediated resistance

M. Fakler[1], S. Löder[1], M. Vogler[1], I. Jeremias[2], K.M. Debatin[1], S. Fulda[1]. [1]*University Children's Hospital, Hematology/Oncology, Ulm, Germany;* [2]*GSF, Gene Vectors, Munich, Germany*

Children with high risk acute lymphoblastic leukemia (ALL) do not respond well to current treatments. This failure is, at least in part, due to defects in apoptosis programs. Therefore, new strategies are required that counter apoptosis resistance in order to improve the poor prognosis of high risk pediatric acute leukemia. Since XIAP, a member of "Inhibitor of Apoptosis Proteins" (IAPs), is expressed at high levels in acute leukemia and blocks apoptosis at a central point of the apoptotic machinery, XIAP may present a suitable molecular target for therapeutic intervention. Here, we report that neutralizing XIAP by small molecule inhibitors is a novel and effective approach to sensitize childhood acute leukemia cells for TRAIL- or chemotherapy-induced apoptosis. XIAP inhibitors at subtoxic concentrations, but not a structurally related control compound, synergize with TRAIL to induce apoptosis in acute lymphoblastic leukemia cells. Also, XIAP inhibitors act in concert with TRAIL to reduce clonogenic growth of ALL cells demonstrating that they suppress long-term survival. Analysis of signaling pathways reveals that XIAP inhibitors enhance TRAIL-induced activation of caspases, loss of mitochondrial membrane potential and cytochrome c release in a caspase-dependent manner. Intriguingly, XIAP inhibitors promote TRAIL-mediated caspase activation, mitochondrial perturbations and apoptosis regardless of high Bcl-2 expression by converting type II leukemia cells that depend on the mitochondrial contribution to the death receptor pathway to type I cells in which activation of effector caspase-3 proceeds irrespective of high Bcl-2 levels. Thus, XIAP inhibitors combined with TRAIL even break Bcl-2-imposed resistance, a defect in the apoptotic pathway that is common in acute leukemia and associated with poor prognosis. Further, XIAP inhibitors prime ALL cells for apoptosis induced by various anti-leukemic drugs, e.g. cytarabine, doxorubicin, etoposide and 6-mercaptopurine, or by agonistic anti-CD95 antibody. In contrast to malignant cells, XIAP inhibitors at equimolar concentrations alone or in combination with TRAIL are non-toxic to normal peripheral blood mononuclear cells despite expression of the apoptosis-inducing TRAIL receptors on the cell surface, pointing to a therapeutic window. Most importantly, XIAP inhibitors enhance TRAIL-induced killing of primary leukemic blasts of children with acute lymphoblastic leukemia. In conclusion, this combined sensitizer/inducer (XIAP inhibitors/TRAIL) strategy is a promising novel therapeutic approach to trigger apoptosis in childhood acute leukemia that warrants further exploitation.

268 POSTER

Chemoresistance in ovarian carcinoma: apoptosis checkpoints in taxol-induced molecular pathways

A. Luria[1], M. Wolfson[1]. [1]*Ben-Gurion University of the Negev, The Shraga Segal Department of Immunology and Microbiology Faculty of Health Sciences Ben-Gurion University of the Negev, Beer-Sheva, Israel*

Background: Ovarian cancer remains one of the most deadly malignancies among gynecological cancers. Chemotherapy which follows cytoreductive surgery is usually based on taxol alone or in combination with platinum compounds. However, resistance to drugs is the main reason for chemotherapy failure. The aim of research is to estimate the correlation between caspase activation and sensitivity to taxol and other compounds used in chemotherapy.
Methods: Using human ovarian carcinoma cell line SKOV3 and primary short living cultures from ascites of ovarian cancer patients, we had evaluated drug-induced apoptosis by cytotoxicity assays, FACS analysis and immunoblotting for apoptosis markers such as caspases and cytochrome *C*.
Results: We have previously reported that taxol-induced apoptosis does not include by caspase-9 activation in SKOV3 and primary ovarian cancer cells *(Ofir et al, 2002. Cell Death Diff. 9, 636–642)*. Here we show that caspases-7 and -10 were strongly activated in taxol-treated cells. Primary ovarian cultures exhibited different sensitivity to taxol treatment. Processing of caspase-7 was found in "taxol-sensitive" cultures and was well correlated with apoptotic death of these cells, whereas no processed caspase-7 and no apoptosis has been found in "taxol-resistant" cells. Interestingly, caspase-10 was processed by taxol in both groups of primary cultures. Preferential activation of caspase-7 by taxol in ovarian cancer cells was specific for this drug, because two other compounds, novobiocin and vinblastin, activated caspase-7 and induced apoptosis in "taxol-resistant" primary cultures.
Since no cytochrome C release and transmembranal potential changes in mitochondria were observed, we assume that mitochondria pathway is not involved in taxol-induced apoptosis. We also conclude that caspase-7 activation could be a useful indicator of susceptibility of ovarian cancer cells to paclitaxel-based therapy.

269 POSTER

Recombinant human epidermal growth factor (rhEGF) inhibits proliferation of cancer cell lines with high level of EGFR expression

E.K. Kwon[1], S.H. Lee[1], H.G. Wu[1]. [1]*Seoul National University College of Medicine, Radiation Oncology, Seoul, Korea*

Background: Epidermal growth factor (EGF) has a role in initiation and progression of cancer, and now EGF receptor inhibitor is utilized in the treatment of some cancers. But we observed and report here cytostatic or cytotoxic effects of EGF in cancer cell lines.
Materials and Methods: Normal fibroblast cell line and two cancer cell lines were used. EGF receptor expression was evaluated using immunoblotting analysis. Two kinds of rhEGF were used in this experiment. Effects of rhEGF on growth of cell lines were assessed by clonogenic assay. rhEGF-induced apoptosis was assessed by flow cytometry analysis (Annecxin-V value), Hoechst staining, and immunoblotting analysis. Effects of combination of rhEGF and ionizing radiation were also evaluated.
Results: rhEGF alone stimulated proliferation of normal fibroblast, but inhibited proliferation of HN3 and A431 cells. Compared to radiation alone, addition of rhEGF attenuated cell killing effect of radiation in normal fibroblast, but it augmented radiation effect in cancer cells. rhEGF induced apoptosis in HN3 and A431 cells with dose- and time-dependent manner and upregulated the expression of cleaved form of caspase 3 correspondingly. Moreover, expression of Ki67 (cell proliferation maker) was significantly decreased in both HN3 and A431 cells with rhEGF, compared to increased expression in normal fibroblast.
Conclusion: Our result suggests that rhEGF could be utilized as anti-cancer treatment in certain cancer cell types such as HN3 and A431 with a high level of EGFR expression.