other known compounds including 3α-acetoxy-25-hydroxyolean-12-en-28-oic acid (**10**), glochidone (**11**) and glochidonol (**12**). Among the cytotoxic compounds, **1**, **5**, **10** and **12** were selective toward MCF-7 cells (IC_{50} 17.1–69.2 microM), whereas compound **7** was more active against DU-145 cells (IC_{50} 20.5 microM).
Conclusions: Ability of some of the compounds in exhibiting selective growth inhibition of breast and prostate cancer cells suggests these agents maybe beneficial in the treatment of human breast and prostate cancers.

185 POSTER

Chlamydocin, a HDAC inhibitor identified by Compare analyses in a cellular screen

H.H. Fiebig[1], T. Metz[1], T. Beckers[1], A. Korrat[1], G. Kelter[1]. [1]*Oncotest GmbH, Institute for experimental Oncology, Freiburg, Germany*

Background: Inhibitors of histone deacetylases (HDAC) were shown to be potent anti-proliferative and pro-apoptotic agents. At Oncotest we have developed a cellular profiling screen in which new compounds are being tested in a standard cell line panel consisting of 36 cell lines from all major solid tumor types with a subsequently Compare Analyses. In the search for novel HDAC inhibitors we have screened a collection of 2000 pure compounds derived from natural products.
Methods: 4,000 to 10,000 adherent cells were seeded in 96 well plates, compounds were added at 5 different concentrations one day later and left over for 4 days. The read-out of the assay is propidium iodide-based fluorescence, which is a measure of viable cell number. Based on IC_{50} and IC_{70} values tumor selectivity of test compounds were analysed. In the Compare Analysis the IC_{50} and IC_{70} pattern of the new compounds are compared with the corresponding patterns of about 100 agents with known mechanism of action using Spearman Correlations.
Results: The known HDAC inhibitors show distinct IC_{50} and IC_{70} activity profile in the Oncotest 36 cell line panel. Concentration-dependent antitumor activity was detected for the 5 structurally diverse HDAC inhibitors Depsipeptide (mean IC_{70} = 0.009 μM), M344 (1.7 μM), SAHA (3.9 μM), acetyldinaline (22 μM) and SBHA (61 μM). The benzamide analog acetyldinaline and M344, as well as the three hydroxamic acids M344, SAHA and SBHA showed similar activity patterns. We used this cellular activity pattern to screen pure natural compounds isolated from bacteria and fungi. Amoung 2000 compounds tested, Chlamydocin showed the closest match with HDAC inhibitors. Chlamydocin was originally isolated from the fungus Diheterospora chlamydosporia. Chemically it belongs to a family of hydrophobic cyclic tetrapeptides. Potent anticancer activity was reported in-vitro. Compare analysis revealed significant correlations of Chlamydocin to M344, SAHA, SBHA and acetyldinaline, the spearman rho ranked between 0.75 and 0.61. Chlamydocin was potent with a mean IC_{70} of 0.018 μg/ml. It showed selective activity in 3/4 prostate, in 3/5 NSCLC, 2/3 ovarian cancer cell lines as well as in 2/5 melanomas.
Conclusion: In conclusion, the evaluation of 5 structurally diverse HDAC inhibitors revealed closely related activity profiles in a panel of 36 cell lines. In the Oncotest cell line screen Chlamydocin was found to be highly potent and selective, and that it act as an HDAC inhibitor a property which was published by Scheper et al (JPET 304:881, 2003). This finding demonstrates that our cellular screen with the subsequent Compare Analysis is able to identify inhibitors against targets of high interest for cancer therapy.

186 POSTER

Screening for the inhibitor against filopodia protrusion

M. Kitagawa[1], E. Tashiro[1], T. Soga[2], M. Imoto[1]. [1]*Keio University, Department of Biosciences and Informatics, Yokohama, Japan;* [2]*Keio University, Institute for Advanced Biosciences, Tsuruoka, Japan*

Background: Filopodia, a rod-like cell membrane protrusion, is a morphological marker of metastatic tumor cells. On the other hand, the use of small molecular inhibitors has potential benefit to dissect the underlying cellular processes in cancer. In this study, we attempted to obtain the inhibitor against filopodia protrusion as analytical tool of metastatic tumor cells.
Material and Methods: Filopodia protrusion: filopodia was induced by Epidermal Growth Factor (EGF) stimulus in human epidermis carcinoma A431 cells. After 30 minutes, filopodia protrusion was observed under microscopy. Screening source or compounds were treated 30 minutes prior to the EGF stimulus. Intracellular ATP: cellular ATP levels were quantitated by ATP assay kit (Sigma). Metabolome analysis: metabolites in cells were collected by methanol extraction. Amounts of each metabolite were quantitatively analyzed by CE-MS system. Glucose uptake: cells were treated with 2-[^{3}H]deoxyglucose, washed and lysed. Radioactivity was counted by Tri-Carb (Perkin-Elmer). And following compounds are additionally used: rotenone, antimycins, and oligomycins.
Results: In the course of screening, we found that glucopiericidin A (GPA) strongly inhibited filopodia in combination with the inhibitors of mitochondrial respiratory chain complexes (MRCIs). Under this condition, we also found that cellular ATP levels were dramatically decreased. Since the process of actin polymerization in filopodia depends on the ATP-energy, it is likely that the decrease of cellular ATP levels caused the inhibition of filopodia protrusion in cells co-treated with GPA and MRCIs. On the other hand, it is well known that inhibition of both glycolysis and mitochondrial oxidative phosphorylation processes results in marked decrease in ATP levels. Thus, we hypothesized that GPA would be a glycolysis inhibitor. To examine this possibility, we conducted metabolome analysis and found that cellular levels of lactate and pyruvate were decreased by the treatment with GPA. Moreover, we found that GPA inhibited cellular incorporation of glucose, indicating that GPA inhibits glycolysis. Meanwhile, malignant tumor cells located within solid tumors possess higher glycolytic capacity because tumors in this region are distant from blood vessels and lack of oxygen, and thereby, mitochondria respiration is limited. This forces them to activate glycolysis to survive. Therefore, we examined whether GPA affects tumor cell viability when mitochondria is suppressed by MRCI. As a result, GPA synergistically induced cell death in A431 cells with MRCI. Therefore, it is likely that GPA, an inhibitor of glycolysis would be effective against the viability in tumor cells.
Conclusions: We identified GPA as a glycolysis inhibitor and suggested that GPA would be a potential candidate for cancer chemotherapy.

187 POSTER

Fusicoccin derivative (ISIR-005) suppresses anchorage-independent growth of cancer cells through anoikis activation

Y. Honma[1], M. Akimoto[1], T. Sassa[2], N. Kato[2]. [1]*Shimane University School of Medicine, Life Science, Izumo, Japan;* [2]*Osaka University, Institute of Scientific and Industrial Research, Osaka, Japan*

Cotylenin A, which has a diterpenoid tricarbocyclic skeleton, was isolated as a plant growth regulator, and has been shown to affect several physiological processes of higher plants and to have differentiation-inducing activity in several myeloid leukemia cell lines. Cotylenin A also affected the differentiation of leukemic cells that were freshly isolated from acute myeloid leukemia patients in primary culture. Injection of the human promyelocytic leukemia cell line NB4 into mice with severe combined immunodeficiency resulted in the death of all mice due to leukemia. Administration of cotylenin A significantly prolonged the survival of mice inoculated with retinoid-sensitive and -resistant NB4 cells without no appreciable adverse effects. Combined treatment with interferon-alpha and cotylenin A significantly inhibited the growth of human lung cancer cells as xenografts without apparent adverse effects. These results suggest that cotylenin A is useful in therapy for leukemia and some other malignancies. However, cotylenin A is difficult to apply to clinical study, since the supply is very limited and it has an epoxide-ring. For clinical application, in the present study, we aimed to synthesize various derivatives from fusicoccins, which are closely related to cotylenin A and are able to be supplied in a large amount as metabolites of phytopathogenic fungus (and examined their differentiation-inducing effects). Although natural fusicoccins did not induce differentiation of myelomonocytic leukemia cells, we synthesized several fusicoccin derivatives with differentiation-inducing activity and without epoxide-ring, based on the structure–activity relationship of cotylenin derivatives. We found some effective derivatives and ISIR-005 was the most potent at inducing differentiation of leukemia cells. Although a low concentration of ISIR-005 hardly affected cell proliferation of lung carcinoma A549 cells, it effectively inhibits anchorage-independent growth and migration of the cells. The drug restored the sensitivity of cancer cells to anoikis. Enhanced anoikis appears to be mediated in part by modulated function of Bcl-2 family proteins.

188 POSTER

Leucinostatins suppress prostate cancer cell growth through the tumour-stromal cell interactions

M. Kawada[1], H. Inoue[1], I. Momose[1], T. Masuda[1], D. IKeda[1]. [1]*Microbial Chemistry Research Center, Numazu Bio-Medical Research Institute, Shizuoka, Japan*

The tumor-stromal cell interactions play an important role in the growth and metastasis of tumors through diffusible factors and cell-cell adhesion. Modulation of the tumor-stromal cell interactions could result in the suppression of tumor growth and metastasis. We have therefore been studying the tumor-stromal cell interactions of prostate cancer and searching for the modulators of the interactions. We designed a coculture system of prostate cancer cells and prostate stromal cells (PrSC) and we recently found that IGF-I secreted from PrSC regulates the growth of prostate cancer. The small molecules that inhibit the growth of prostate cancer cells in coculture with PrSC will become new type anticancer

drugs modulating the tumor-stromal cell interactions. In this study we have reported the results of our screening of the small molecules using microbial cultured broth. We purified the active small molecules and identified them as leucinostatins and atpenins. They significantly inhibited the growth of prostate cancer DU-145 cells in coculture with PrSC, but they only slightly affected that in monoculture. We evaluated their antitumor effects using a mouse xenograft model of coinoculation of DU-145 and PrSC. As a result, leucinostatin A suppressed the growth of DU-145 tumors more significantly than atpenin B did. Leucinostatin A was found to inhibit the expression of IGF-I in PrSC. Since these small molecules are considered to modulate the tumor-stromal cell interactions of prostate, we are now studying the mechanism of their actions.

189 POSTER

Solution structure of a 2:1 C2-(2-naphthyl)pyrrolo[2,1-c][1,4]-benzodiazepine (PBD) DNA adduct: molecular basis for unexpectedly high DNA helix stabilization

D. Antonow[1], T. Barata[1], T.C. Jenkins[2], G.N. Parkinson[1], P.W. Howard[3], M. Zloh[1], D.E. Thurston[1]. *[1]School of Pharmacy, University of London, London, United Kingdom; [2]Morvus Technology Ltd, Porton Down Science Park, United Kingdom; [3]Spirogen Ltd, London, United Kingdom*

The naturally occurring PBD monomers such as sibiromycin, anthramycin and tomaymycin form stable covalent adducts with duplex DNA at purine-guanine-purine sequences. A correlative relationship exists between DNA-binding affinity (as measured by enhanced thermal denaturation of calf-thymus DNA; ΔTm) and cytotoxicity for these naturally occurring compounds and a range of synthetic analogues. Sibiromycin has the highest ΔTm value (16.3ºC) of the naturally occurring PBDs reflecting a number of favourable hydrogen bonding interactions between the molecule and DNA bases. We report here that, surprisingly, the simple synthetic C2-(2-naphthyl)-substituted pyrrolo[2,1-c][1,4]benzodiazepine monomer SG2313 (DA046) provides a ΔTm value (15.8ºC) of the same order of magnitude as sibiromycin and significantly higher than those for anthramycin (13.0ºC) or tomaymycin (2.6ºC). It is also similar in cytotoxic potency to sibiromycin which is widely regarded as the most potent naturally occurring PBD monomer. Given the structural simplicity of SG2313 compared to sibiromycin, we have investigated its unexpectedly high ΔTm using high-field NMR in conjunction with molecular dynamics to study its interaction with the DNA duplex d(AATCTTTAAAGATT)2. A 2:1 drug/DNA adduct was observed similar to that reported by Hurley et al for tomaymycin. The results show that the high binding affinity of SG2313 is due predominantly to hydrophobic (van der Waals) interactions in contrast to the hydrogen bonds which predominate in the case of sibiromycin, anthramycin and tomaymycin drug/DNA adducts. Using high resolution 2D NOESY experiments, unequivocal determination of the orientation of the SG2313 molecule (i.e., A-ring towards 3′-end of covalently bound strand) and stereochemistry at the C11-position (C11S) could be achieved. In addition, the location of the C2-naphthyl ring could be determined, indicating that it extends along the floor of the minor groove thus optimizing hydrophobic interactions with DNA functional groups and explaining the high ΔTm value. These results provide further opportunities for drug design in terms of extending planar hydrophobic groups at the C2-position of PBDs to maximize DNA binding affinity.

Paediatric – early drug development

190 POSTER

Pediatric Preclinical Testing Program (PPTP) evaluation of rapamycin combined with cytotoxic drugs used frequently in treatment of childhood cancer

P.J. Houghton[1], C.L. Morton[1], J.M. Maris[2], S.T. Keir[3], R.B. Lock[4], H. Carol[4], R. Gorlick[5], E.A. Kolb[5], M.H. Kang[6], M.A. Smith[7]. *[1]St Jude Children's Research Hospital, Molecular Pharmacology, Memphis, USA; [2]Children's Hospital of Philadelphia, Oncology, Philadelphia, USA; [3]Duke University Medical Center, Oncology, Durham, USA; [4]Children's Cancer Institute, Oncology, Randwick, Australia; [5]Albert Einstein College, Oncology, Bronx, USA; [6]Children's Hospital of Los Angeles, Oncology, Los Angeles, CA, USA; [7]National Cancer Institute, Cancer Treatment Evaluation Program, Bethesda, USA*

Background: Rapamycin (Rap) is a specific inhibitor of mTOR that has demonstrated broad-spectrum antitumor activity as a single agent against the PPTP in vivo panels of childhood tumors. Here we have extended the studies with Rap to combinations with agents used frequently in the treatment of childhood malignancies.
Methods: Rap was tested against the PPTP in vitro panel of 23 cell lines at a concentration of 10 nM alone or in combination with increasing concentrations of melphalan, cisplatin, vincristine or dexamethasone [acute lymphoblastic leukemia (ALL) models only]. Rap was tested in vivo at a dose of 5 mg/kg i.p. 5 days per week for 6 weeks for solid tumors or 4 weeks for leukemia models. Cytotoxic agents were administered at their maximum tolerated dose (MTD, approximately LD10), and 0.5 $\times$ MTD. Three measures of antitumor activity were used: (1) response criteria modeled after the clinical setting; (2) treated to control (T/C) tumor volume at day 21; and (3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines.
Results: Combining Rap with cytotoxic agents in vitro gave predominantly <-additive or additive effects, except with dexamethasone in ALL models for which the effect was >-additive. In vivo Rap significantly increased the toxicity of cisplatin but not vincristine or cyclophosphamide. Rap combined with vincristine (MTD) was additive or >-additive in 10 of 12 models and with cyclophosphamide (MTD) was additive or >-additive activity in 8 of 9 models and antagonistic in 1 model. Cisplatin (0.63 $\times$ MTD) – Rap combination gave additive or >-additive activity in 9 of 9 models. Against the ALL panel the combination with vincristine was predominantly <-additive, while with cyclophosphamide the effect was additive or <-additive. Rap combined with dexamethasone was >-additive, additive, or antagonistic, respectively, in 3 ALL models.
Conclusions: Rap combined with cyclophosphamide or vincristine appeared superior to either single agent against several tumor models. There was little evidence that rapamycin potentiated the toxicity of these agents. Rap significantly potentiated the toxicity of cisplatin. However, the antitumor activity of Rap combined with either cisplatin administered at 0.63 $\times$ MTD or with vincristine or cyclophosphamide (both at 0.5 $\times$ MTD) was greater than that for each cytotoxic agent alone administered at its MTD in most solid tumor models.
(Supported by NCI NO1CM42216)

191 POSTER

Pediatric Preclinical Testing Program (PPTP) evaluation of the oncolytic picornavirus, NTX-010 (SVV-001)

C.L. Morton[1], P.J. Houghton[1], J.M. Maris[2], R. Gorlick[3], E.A. Kolb[4], M.H. Kang[5], C.P. Reynolds[5], M.A. Smith[6]. *[1]St. Jude Children's Research Hospital, Molecular Pharmacology MS230, Memphis, TN, USA; [2]Children's Hospital of Philadelphia, Pediatrics, Philadephia, PA, USA; [3]Albert Einstein College of Medicine, Pediatrics, Bronx, NY, USA; [4]A.I. duPont Hospital for Children, Pediatrics, Wilmington, DE, USA; [5]Children's Hospital of Los Angeles, Pediatrics, Los Angeles, CA, USA; [6]National Cancer Institute, Cancer Therapy and Evaluation Program, Bethesda, MD, USA*

Background: NTX-010 is a novel oncolytic picornavirus with antitumor activity against human cancers expressing neuroendocrine markers. The activity of NTX-010 was evaluated against the in vitro and in vivo panels of the Pediatric Preclinical Testing Program (PPTP).
Methods: The PPTP includes a molecularly characterized in vitro panel of cell lines (n = 27) and in vivo panel of xenografts (n = 61) representing most of the common types of childhood solid tumors and childhood acute lymphoblastic leukemia (ALL). NTX-010 was tested against the PPTP in vitro panel at concentrations ranging from 10^4 virus particles per cell to 10^{-4} virus particles per cell and was tested against the PPTP in vivo panels at a dose of 3×10^{12} virus particles per kg administered as a single dose via intravenous injection. Three measures of antitumor activity were used: (1) response criteria modeled after the clinical setting; (2) treated to control (T/C) tumor volume at day 21; and (3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: NTX-010 was variably active against lines in the in vitro panel with activity focused in the Ewing, neuroblastoma and rhabdomyosarcoma histologies, while no activity was observed against leukemia and lymphoma lines. NTX-010 achieved objective responses in 12 of 35 xenografts tested (34%) with objective responses in the Wilms, rhabdoid, glioblastoma, neuroblastoma and alveolar rhabdomyosarcoma panels. Activity was greatest for the neuroblastoma and alveolar rhabdomyosarcoma panels. In the neuroblastoma panel, there was 1 partial response and 3 maintained complete responses (MCRs) among 5 xenografts tested. Each of the 4 alveolar rhabdomyosarcoma xenografts tested achieved MCRs. NTX-010 was not evaluated against the ALL in vivo panel.
Conclusions: NTX-010 demonstrated activity against the PPTP's in vitro and in vivo solid tumor panels, with activity concentrated in models expressing neuroendocrine markers (e.g., NCAM1). Particularly notable was the high level of in vivo activity observed for the neuroblastoma and alveolar rhabdomyosarcoma panels. Further studies characterizing molecular predictors of response and the activity of combinations of NTX-010 with other anticancer agents are anticipated.
(Supported by NCI NO1CM42216)