clinical results. Finally, we simulated putative treatment schedules for identifying a possible effective treatment for GBM.
Results: Our simulation results successfully reproduce the experimental results of Kruse *et al*, (2001) for BTs grade III. Our model suggests several alternative schedules and dosages that manage to destroy the tumor. For a patient who died from a recurrence of BT grade III tumor, our model predicts that a longer treatment course may have been required to prevent tumor resurgence. The model interprets the failure of immunotherapy in the case of BT grade IV and predicts that a more intensive treatment protocol could eradicate GBM. We suggest alternative treatment courses for the eradication of GBM.
Conclusions: CTL immunotherapy is an effective therapy for BT grade III and IV. It can be optimized to prevent tumor recurrence. We believe that the experimental failure of Kruse *et al.* (2001) to treat GBM patients originated from immunotherapy not sufficiently intensive to overcome such a highly aggressive tumor (for example, using the same CTL dose we suggest daily infusions instead of every 4–5 days).

References

Kruse CA, Rubestein D. 2001. In Liau LM, Becker DP, Cloughesy TF, Bigner DD (Eds), *Brain Tumor Immunotherapy.* Humana Press, Totowa, NJ. pp149–170.

273 POSTER

Candidate tumor suppressor gene DLEC1 on 3p21.3 is hypermethylated in hepatocellular carcinoma

G.H. Qiu[1], N. Wheelhouse[2], D. Harrison[2], G.G. Chen[3], S. Manuel[1], P. Lai[3], J.A. Ross[2], S.C. Hooi[1]. *[1]National University of Singapore, Singapore, Singapore; [2]University of Edinburgh, Edinburgh, UK; [3]The Chinese University of Hong Kong, Hong Kong, China*

Background: DLEC1 (previously also known as DLC1) is located at 3p21.3, which is one of the most frequent LOH regions in human chromosomes. It encodes a 1755-amino acid polypeptide and is localized only in the cytoplasm, with no homology to any known protein or domain. DLEC1 has been shown to have tumor suppressor function in cancer cell lines by colony formation assay. However, no alteration of the gene has been detected to cause dysfunction of its product in any of the cancers examined. Nevertheless, a CpG island has been found in the region of its promoter and first exon. Therefore, we tested the hypothesis that methylation of DLEC1 might suppress its expression to inactivate this tumor suppressor gene in hepatocellular carcinoma (HCC).
Material and Methods: HCC cell lines Hep3B, HepG2, Chang Liver, PLC/PRF/5 and SK-Hep-1, and 57 pairs of HCC primary tumors and matched adjacent normal samples were used. DNA methylation was detected by MSP and expression level by RT-PCR. Transfection of cell lines was mediated by Lipofectamine 2000 and transfected cells were selected by G418.
Results: DLEC1 is methylated in HCC cell lines Hep3B, HepG2, Chang Liver, PLC/PRF/5 and SK-Hep-1. The treatment of these cell lines with 5-aza-2'-deoxycytidine restored its expression. Using real-time RT-PCR and HCC primary tissues, we found that the expression level of DLEC1 in tumor samples was significantly lower than that in matched adjacent normal samples (t test, $p < 0.05$). Similarly, expression of DLEC1 in methylated samples was also significantly lower than that in unmethylated samples (t test, $p < 0.05$). Moreover, hypermethylation of DLEC1 was detected in 40 of 59 (67.8 %) primary tumors, while only 6 in 57 (10.5 %) nonmalignant specimens ($p < 0.001$, chi-square). We examined the relationship between DLEC1 methylation status and clinicopathological features, including age, gender, alpha-fetoprotein (AFP) levels, tumor size, ALTSG stage, AJCC stage, differentiation status, cirrhosis, encapsulation, vascular and capsule invasion in 49 samples with their tumor stages identified. The DLEC1 methylation status was associated with AJCC stages of tumors ($p = 0.036$, chi-square). Colony formation assay of exogenous expression of DLEC1 in cell lines showed that DLEC1 significantly inhibited cancer cells growth.
Conclusions: Our data showed that *DLEC1* is hypermethylated in the majority of hepatocellular carcinoma and able to suppress the growth of liver cancer cell lines, supporting its role as a tumor suppressor.

274 POSTER

Effect of IFN-α on gene expression: cDNA microarray analysis in human epidermoid cancer cells

G. Giuberti[1], M. Marra[1], A.M. D'Alessandro[1], A. Lombardi[1], D. Vozzi[2], P. Gasparini[2], A. Abbruzzese[1], A. Baldi[1], M. Caraglia[1]. *[1]Second University of Naples, Department of Biochemistry and Biophysics, Naples, Italy; [2]Second University of Naples, Department of Pathology, Naples, Italy*

Introduction: Interferon-alpha a cytokine commonly used in the human cancer therapy, our research group is deeply involved in the characterization of the effects of IFNalpha activity on tumor cells. In details, we have found that IFN induces into human epidermoid cancer cells KB apoptosis and upregulates the expression of the Epidermal Growth Factor Receptor (EGFR) [1,2].
Materials and Methods: RNA Extraction; Probe Synthesis; Hybridization on cDNA Arrays; Statistical Analysis; Northern blot analysis; Quantitative Real Time PCR, Western blotting.
Results: In order to better characterize the molecular pathways that are elicited or suppressed by the action of IFN. A human 1.7k microarray (Microarray centre U.H.N., Canada) array was used for this experiment, which allows the simultaneous analysis of more than 1.5 thousand genes. Analysis of the hybridization signals through the use of a dedicated software (SAM and processed and analysed with MIDAS), has identified 25 differentially expressed genes: 19 down-regulated and 6 up-regulated in KB cells treated with IFN: prenylcysteine lyase:PCL, tissue inhibitor of met-alloproteinase 3 (Sorsby fundus dystrophy, pseudoinflammatory):TIMP3, proteasome (prosome, macropain) activator subunit 1 (PA28 alpha), guanine monophosphate synthetase:GMPS. The differential expression of these genes in the two cell lines is being confirmed by northern blot analysis and quantitative RT-PCR as like the proteins expression.
Conclusions: This study represents a useful basis to define an extended molecular database of potential relevance both in basic cell biology studies and in therapeutic options.

References

[1] M. Caraglia et al., (1999) Cell Death Differ 6: 773–780
[2] M. Caraglia et al., (2003) Cell Death Differ. 10(2):218−29.
[3] M. Boccellino et al., (2004) J Cell Physiol, in press
[4] C. Esposito et al., (2003) Biochem. J. 370, 205–212
[5] A. Baldi et al., (2002) J Cell Physiol.;193(2):145−53. Review.

275 POSTER

Tumour cells from stage III melanoma patients are often resistant to growth inhibition by Oncostatin M

A. Lacreusette[1], J. Nguyen[2], M. Pandolfino[1], A. Khammari[3], B. Dreno[3], Y. Jacques[1], A. Godard[1], F. Blanchard[4]. *[1]INSERM U601, Cancerology department, Nantes, France; [2]PIMESP, CHU, Nantes, France; [3]Unit of Skin Cancer, CHU, Nantes, France; [4]INSERM ERI7, Nantes, France*

Oncostatin M (OSM) is an Interleukin-6 (IL-6) type cytokine originally described by its capacity to inhibit melanoma proliferation in vitro. However, OSM responsiveness is often lost in advanced stages melanoma cells. Here, the mechanisms involved in resistance to growth inhibition by OSM and IL-6 were analyzed for the first time on a large panel of metastatic melanoma cell lines (35). For 28% of the cell lines, OSM resistance correlated with the epigenetic loss of the OSM receptor beta (OSMRb) subunit. Treatment of these cells with the histone deacetylase inhibitor Trichostatin A re-established histone acetylation in the OSMRb promoter, expression of OSMRb and growth inhibition by OSM. Other defects linked to OSM resistance were identified, for 31% of the cell lines, on specific signal transduction pathways such as STAT3 (Ser727 phosphorylation), PKCa/b/d and/or AKT, explaining their co-resistance to OSM and IL-6. The use of PKCa/b/d inhibitors indicated that these serine kinases, together with STAT3, have a crucial role in growth inhibition by OSM. In nude mice injected with sensitive melanoma cell lines, OSM notably reduced tumour growth. Moreover, the patients whose melanoma cells were sensitive to growth inhibition by OSM and/or IL-6, and who were treated with tumor-infiltrating lymphocytes (as a potent source for these cytokines; $n = 13$), have a mean relapse-free survival of 8 years. Those whose melanoma cells were resistant to these cytokines ($n = 6$), have a mean relapse-free of only 15 months. Altogether, our results suggest a role for OSM in the prevention of melanoma progression in vitro and in vivo, and that metastatic melanoma cells could escape this growth control by the loss of OSMRb or defects on specific signal transduction pathways. We are currently validating on larger cohorts of patients, the involvement of IL-6 type cytokines in the response to immunotherapy and looking for a specific inflammatory state that could induce this cytokine resistance.

276 POSTER

The frizzled 8-related antiproliferative factor from IC patients inhibits bladder and kidney carcinoma cell proliferation in vitro

S. Keay[1], G. Tocci[2], K. Koch[1], C. Zhang[3], D. Grkovic[1], C. Michejda[2]. *[1]University of Maryland School of Medicine and VA Medical Center, Medicine, Baltimore, MD, USA; [2]National Cancer Institute, Center for Cancer Research, Frederick, MD, USA; [3]University of Maryland School of Medicine and VA Medical Center, Pathology, Baltimore, MD, USA*

Background: Antiproliferative factor (APF) is a potent sialoglycopeptide inhibitor of epithelial cell proliferation made by bladder cells from patients

with interstitial cystitis (IC), a disease characterized by thinning or ulceration of the bladder epithelium. The peptide chain of APF has 100% homology to the 6th transmembrane domain of frizzled 8, a Wnt ligand receptor. Because increased Wnt expression and TCF-dependent promoter activation by β-catenin are associated with human urinary bladder carcinoma, and decreased expression of E-cadherin (a negative regulator of β-catenin) is associated with invasiveness of bladder carcinoma cells, Wnt signaling may play an important role in bladder cancer. Early studies indicated that purified native APF increased E-cadherin expression and decreased proliferation of bladder epithelial cells *in vitro* at high picomolar concentrations, and both native and synthetic APF were shown to inhibit normal bladder epithelial as well as bladder cancer (T24) cell proliferation *in vitro* at high picomolar concentrations. We therefore determined whether APF is also active against cell lines derived from other urologic carcinomas.
Materials and Methods: Two bladder cancer lines (TCC-SUP and SCaBER) and two kidney cancer lines (A498 and ACHN) were incubated with varying concentrations of synthetic APF or the inactive unglycosylated peptide for 48 hrs prior to determination of cell growth by live cell count and/or ^{3}H-thymidine incorporation.
Results: All four cell lines derived from bladder or kidney malignancies (TCC-SUP, SCaBER, A498 and ACHN) proved to be as sensitive to APF as normal bladder epithelial and T24 cells, having an IC_{50} in the low (0.25−2.5) nanomolar range. In comparison, none of these cell lines was inhibited by the inactive unglycosylated backbone peptide.
Conclusions: APF appears to be a potent inhibitor of both normal bladder epithelial cell and urologic cancer cell proliferation, and its ability to stimulate E-cadherin expression may also be useful for inhibiting bladder cancer invasion. Studies in progress will determine whether carcinomas from tissues other than the urinary tract are also sensitive to APF, as well as correlate its effects on cell proliferation with cellular production and localization of E-cadherin and β-catenin.

277 POSTER

An interleukin-6 antagonist modified for bone targeting preserves anti-myeloma biological activity

F. Casadonte[1], R. Terracciano[1], M. Gaspari[1], R. Arcone[2], N. Malara[1], D. Paolino[2], M. Fresta[2], P. Tassone[1], S. Venuta[1], R. Savino[1]. *[1]"Magna Graecia"' University, Experimental and Clinical Medicine, Catanzaro, Italy; [2]"Magna Graecia" University, Pharmacia, Catanzaro, Italy*

Multiple Myeloma (MM) is a malignancy affecting terminally differentiated B-cells. In MM, malignant plasma cell clones are predominantly localized in the bone marrow microenvironment, due to adherence both to extra cellular matrix proteins and to bone marrow stroma cells (BMSCs). This interaction, between tumor cells and BMSC, triggers production of cytokines mediating growth and survival of MM cells. Interleukin-6 (IL-6) has been demonstrated to be a major growth factor in MM, as it is responsible for both autocrine and paracrine growth induction and for preventing apoptosis of human myeloma cells. We generated an IL-6 receptor antagonist, Sant7, which acts as growth inhibitor and enhances apoptosis on human MM by sequestering the IL-6 receptor alpha and preventing wild type IL-6 signalling. However, when injected in experimental animals, Sant7 has a very fast pharmacokinetic, reaching effective concentrations in the BM microenvironment only for limited periods. Recent reports demonstrated that conjugation with amino-bisphosphonate (ABP) targets systemically administered proteins to the bone of experimental animals. To target the IL-6 antagonist to BM microenvironment we tested the conjugation with ABP. Sant7-ABP conjugation required first the conversion of the amino group of ABP to thiol group using Traut's reagent. The thiolated ABP was then treated with the hetero-bifunctional linker SMCC (Sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate) and the resulting reaction mixture was added to the protein solution. The conjugated product was analysed by MALDI-TOF mass spectrometry (Matrix Assisted Laser Desorption Ionization-Time of Flight). Mass shift of conjugation product respect to unconjugated protein suggested 1 to 6 molecules of ABP were conjugated to Sant7, depending on the reaction conditions. The biological activity of the Sant7 preparation was tested performing growth inhibition assay on INA-6 IL-6 dependent human MM cell lines. The growth inhibition assay on INA-6 IL-6 dependent human MM cell line demonstrated that the Sant7-ABP product is biologically active, able to inhibit the activity of IL-6 induced INA-6 proliferation. Furthermore we demonstrated that the inhibition is not due to the ABP moiety. Our results suggest that the Sant7-APB conjugate can be used as effective therapeutic agent against the MM due to potential specific bone marrow targeting.

278 POSTER

Selective Aurora A inhibitors – in vitro potency, specificity and cellular mode of action

D. Zheleva, F. Scaerou, J. McLachlan, G. Wood, D. Sorrel, D. Jones, W. Jackson, K. Duncan, G. Griffiths, S. Jones. *Cyclacel Ltd, Polgen Division, Cambridge, United Kingdom*

The mitotic kinase Aurora A plays critical roles in centrosome function, bipolar spindle formation and chromosome segregation. Aberrant expression or activity of this serine/threonine protein kinase has been implicated in tumorigenesis and resistance to Taxol, making it an attractive anticancer target. To better define the inhibitory properties of our candidate aurora inhibitors, we have carried out a detailed comparison of the effects of inhibiting or knocking down Aurora A and/or Aurora B using small molecules or siRNA treatment respectively. We have identified a proprietary series of potent Aurora A inhibitors. These small molecules possess various degrees of selectivity for Aurora A versus Aurora B and are highly selective for the Aurora kinase family over a panel of more than 50 kinases. The observed cellular mode of action is consistent with selective Aurora A inhibition – monopolar spindle formation, delayed progression through mitosis, perturbation of Aurora A specific markers and apoptosis induction. Induction of p53 transcriptional activity and caspase activity is also observed. The antiproliferative activity of Aurora A selective inhibitors has been evaluated in a panel of tumour cell lines using a standard MTT cytotoxicity assay and a colony formation assay. The cellular mode of action and the PK properties of several members of the class studied in rodents (high oral bioavailability and half life) suggest the suitability of this series for further development. The rationale for the development of Aurora A specific inhibitors will also be discussed.

Cyclins and CDKs

279 POSTER

Pharmacodynamic effects of seliciclib (r-roscovitine, cyc202) in patients with undifferentiated nasopharyngeal cancer (NPC) using a window trial design

W.S. Hsieh[1], B.K. Peh[2], T. Loh[2], J.H. Chiao[3], R. Soo[4], S.R. Green[3], Y.F. Lai[1], M. Salto-Tellez[2], R. Soong[2], B.C. Goh[4]. *[1]Division of Biomedical Sciences Johns Hopkins in, Singapore, Singapore; [2]National University of Singapore, Singapore; [3]Cyclacel Ltd, Dundee, United Kingdom; [4]National University Hospital, Singapore*

Cell cycle dysregulation, characterized by inactivation of cyclin dependent kinase (CDK) inhibitors, e.g., p16 and p27, and over-expression of cyclin D1 is frequently found in undifferentiated NPC. Loss of either p16 or p27 or over-expression of cyclin D1 leads to increased Rb phosphorylation, resulting in uncontrolled cellular proliferation. Seliciclib, an orally administered inhibitor of CDKs (including 2, 7 and 9) inhibits phosphorylation of both Rb and RNA polymerase II and induces apoptosis in several human tumor cell lines including NPC cells lines through the inhibition of transcription leading to a reduction in the levels of key anti-apoptotic proteins like Mcl-1, survivin and XIAP. We evaluated the clinical, cellular and molecular effects of seliciclib in 16 patients with chemonaive NPC. Tumor biopsies and plasma were taken pre- and post-treatment (day 12) with suitable paired samples being obtained in 14 of the 16 treated patients. Of 14 evaluable patients, 7 had >25% reduction in clinically measurable cervical lymph nodes. Histologic examination of post-treatment biopsy samples showed increased tumor necrosis and immunohistochemical staining showed increased tumor cell apoptosis and decrease in Mcl-1 and cyclin D1. Tumor RNA was subjected to a low density real time PCR array consisting of a set of 384 genes related to cell cycle, apoptosis, and cell signaling. The results confirmed known biological effect of seliciclib in terms of transcriptional inhibition. Seven of 14 tumor biopsy pairs showed greater than 25% decrease in RNA levels of at least 25% of genes studied while 5 of these 7 pairs showed greater than 50% decrease in RNA levels of at least 25% of the genes examined. Biological effects attributable to seliciclib were also seen in plasma. An increase in cell death markers circulating in plasma was seen in 4/14 patients. Serum EBV copy number, a marker of disease burden in NPC, was reduced by at least 50% in 4/13 patients. In summary, seliciclib has clinical antitumor activity against NPC at a dose that is well tolerated. The clinical effect is accompanied by biological effects in tumor tissue consistent with the known mechanism of the agent. Effects observed included decreases in transcription and decreases in some cell cycle related proteins and anti-apoptotic proteins. These effects were correlated with a reduction in serum EBV copy number and an increase in plasma biomarker consistent with tumor cell death.