348 POSTER

Targeting pancreatic cancers with a G-quadruplex binding small molecule

S.M. Hampel[1], M. de la Fuente[2], A. Schatzlein[2], A. Sidibe[3], J.F. Riou[3], S. Neidle[1]. *[1]The School of Pharmacy, Cancer Research UK BMSG, London, United Kingdom; [2]The School of Pharmacy, Cancer Pharmacology Group, London, United Kingdom; [3]Museum National d'Histoire Naturelle, Regulation et Dynamique des Genomes, Paris, France*

We have previously reported on a novel series of tetraaminoalkyl substituted naphthalene diimide compounds that bind to G-quadruplex nucleic acids and have high potency against a panel of human cancer cell lines.
We now report on a further subset of highly water soluble derivatives with methyl piperazine end groups, which show a high affinity and selectivity for human telomeric G-quadruplexes over duplex DNA. These compounds are similarly potent in the cancer cell line panel, and also show potency against several pancreatic cancer cell lines, with typical IC_{50} values in the dimension of 0.1 μM. The compounds have >40-fold selectivity over a normal fibroblast cell line. Treatment with the compounds at sub cytotoxic concentrations over several weeks lead to a decrease in growth of pancreatic cancer cells, and they stained positive for senescence. *In vitro* experiments show that the compounds inhibit the binding of hPOT1 and TopoisomeraseIIα to G-quadruplex DNA. The fluorescent compounds were visualised inside pancreatic cancer cells by confocal microscopy. In a flow cytometry experiment the phosphorylated Histone H2A.X (Ser139), which occurs in response to DNA damage, was detected in MIA-Pa-Ca-2 cells incubated with the compounds for 14 h. Cell cycle analysis with Propidium Iodide showed an increase of cells in G2/M phase. One of the compounds was evaluated in an *in vivo* study using the pancreatic cancer MIA-Pa-Ca-2 xenograft model. It was well-tolerated when dosed 3 mg/kg every 48 h by intraperitoneal injection. The anti cancer activity of the compound is displayed in form of growth delay of the tumors in the treated animals. The results of the preliminary evaluation of the reported compounds including investigations in their mechanism of action and the *in vivo* study suggest that they are good candidates for G-quadruplex binding anti cancer drugs.

349 POSTER

Monocarboxylate transporter 1 as a potential therapeutic target in glioblastomas

V. Gonçalves[1], C. Pinheiro[1], M. Honavar[2], P. Costa[3], R. Reis[1], F. Baltazar[1]. *[1]Life and Health Sciences Research Institute (ICVS), Surgical Sciences, Braga, Portugal; [2]Hospital Pedro Hispano, Pathology, Matosinhos, Portugal; [3]CUF Institute, CUF Institute, Porto, Portugal*

Background: Glioblastoma is the brain tumor with the highest prevalence and lethality worldwide, having a very low overall survival. Therefore, it is imperative to study new molecular therapeutic targets that could improve the life time of these patients. Dependence of aerobic glycolytic metabolism is a characteristic of malignant tumours, including glioblastomas. To allow continuous glycolysis and prevent cell death from increased intracellular acid accumulation, tumour cells upregulate pH regulators, such as monocarboxylate transporters (MCTs). The efflux of acid lactic produced by glycolysis is dependent of MCTs, which facilitate the co-transport of short-chain fatty acids such as lactic acid, coupled with a proton (proton symport). Expression of MCTs has been described in same tumours, however studies in brain tumours are scarce. Therefore, we aimed to characterize the expression of MCT1, MCT4 and their chaperone protein (CD147) in glioblastoma human samples, as well as in tumour cell lines. Further, we aimed to assess the sensitivity of glioma cell lines to MCT inhibitors.
Methodology: Expression of MCT1 and MCT4, as well as their chaperone protein CD147 was evaluated by immunohystochemistry in 78 cases of glioblastoma samples and in 8 different glioblastoma cell lines by immunohystochemistry and western blot. Sensitivity of these cell lines to MCT inhibitors was assessed using viability/proliferation assays.
Results: MCT1 and CD147 expressions were increased in glioblastomas tissues, compared to the non-neoplastic tissue. A variety of glioma cell lines expressed both MCT1 and MCT4 isoforms, although with different cellular localizations. In most glioma cell lines, both MCT1 and CD147 were expressed in the plasma membrane, while MCT4 expression was only detected in the cytoplasm. Further, MCT classical inhibitors significantly decreased the viability/proliferation of glioma cell lines.
Conclusions: These data provide evidence that MCT1 might play an important role in glioblastoma survival. Thus, exploitation of MCT inhibitors may represent promising strategy in glioblastoma therapy.

350 POSTER

Target therapy and endoarterial chemotherapy with ozonotherapy in combined treatment of metastatic colorectal cancer

E.T. Akbarov[1], S.N. Navruzov[1], S.B. Abdujapparov[1], H.D. Islamov[1]. *[1]National Cancer Research Center of Uzbekistan, Coloproctology, Tashkent, Uzbekistan*

Background: Improvement of quality and increasing of longevity of metastatic colorectal cancer we used endoarterial infusion combined with target therapy, immunotherapy and ozonotherapy.
Materials and Methods: We have taken randomized study on 68 patients in period during 2006–2010 year from them in 1st group (23 patients) included patients that have taken Bevacizumab (5 mg/kg intravenous infusion every 7 days), immunomodulator by intramuscular injection of Transfer Albuminatus Factor (TAF) and intraarterial infusion of ozonized liquor with chemotherapy (CT) by scheme FOLFOX-4 (Oxaliplatin-100 mg/m^2 on 1st day, Leukovorin-200 mg/m^2 on 1st day, 5 Fluorouracil (5FU)-400 mg/m^2 intraarterial intermittent administration on 1st day, then 5FU − 2.4–3.0 g/m^2 48 hours flat continuous infusion) with interval 3 weeks. 2nd group (20 patients) taking CT by similar scheme of intravenous infusion withoutimmunotherapy and target therapy, with interval 3 weeks.
Results: All patients analyzed for toxicity. Main G 2−3 side-effects were: neutropenia on I group − 4.3%, II group − 50%, diarrhea − 4.3; 20%, stomatitis − 4.3%; 10%, neurotoxity − 0; 5%, deep venous thrombosis − 0%; 5%, hypertension − 4.3%; 15%, cardiac ischemia − 0; 5%.No toxic deaths have occurred. All patients have been evaluated for response and we observed 7 cases in I group (1 in II group) complete and 13 (15 in II) partial response rate and 3 stable disease (4 in II group). Up to now 18 patients in I group and 6 in II underwent post-CT surgical resection of metastases with curative intent and 14; 2 − R0 resection have been performed. At median follow-up of 16.3 and 11.8 months, 9 and 18 patients have progressed and median progression-free survival (PFS) is 13.1; 9.2 months with an actual PFS at 10 months of 72%; 43%. To date 7; 16 patients have died and median overall survival (OS) has not yet been reached.
Conclusion: Using endoarterial chemotherapy can be safely combined with ozonotherapy, immunotherapy and target therapy without increasing toxicities no causing unforeseen adverse events. Preliminary data in terms of RR, secondary resection of metastases and PFS are promising increase effect of endoarterial CT.

351 POSTER

Inhibition of monocarboxylate transporter 1 in cervical cancer cells: effect of alpha-cyano-4-hydroxycinnamate and siRNA

C. Pinheiro[1], S. Pinheiro[1], E. Bocardo[2], A.P. Lepique[2], A. Longatto-Filho[3], L.L. Villa[2], F. Baltazar[1]. *[1]Life and Health Sciences Research Institute (ICVS), School of Health Sciences University of Minho, Braga, Portugal; [2]Ludwig Institute for Cancer Research, (LICR), Sao Paulo, Brazil; [3]Laboratory of Medical Investigation (LIM-14), School of Medicine Sao Paulo University, Sao Paulo, Brazil*

Background: Up-regulation of glycolytic metabolism, even in the presence of oxygen (Warburg effect), has been described as a possible adaptive mechanism to overcome intermittent hypoxia in pre-malignant lesions. In this context, monocarboxylate transporters (MCTs) emerge as important players due to their dual function, as lactate exporters, allowing continuous glycolysis, and tumour intracellular pH regulators, by co-transporting lactate and a proton, inducing extracellular acidosis. We have recently described the up-regulation of monocarboxylate transporter 1 (MCT1) along the progression towards invasive cervical carcinoma. Therefore, we aimed to evaluate the effect of MCT1 inhibition in cervical cancer cells.
Material and Methods: The metabolic behaviour of the human cervical cancer cell lines SiHa, CaSki, HeLa, Sw756, C33 and HaCat, with different high-risk HPV status, was characterised. Extracellular lactate and glucose were quantified using commercial kits. MCT1 activity was inhibited in HeLa cells using the classical MCT inhibitor alpha-cyano-4-hydroxycinnamate (CHC) while MCT1 expression was silenced using siRNA. The inhibitory effects were estimated using the Sulforhodamine B assay. Statistical analysis was performed using the SPSS statistical software.
Results: Glucose consumption and lactate production varied among cell lines, with CaSki, HeLa and HaCat being the most glycolytic cell lines and SiHa showing the lowest rates of glucose consumption and lactate production. Hence, HeLa was further used to perform MCT1 inhibition studies. When exposing cells to CHC, we found a significant decrease in total cell biomass, with an IC_{50} value of 7.29 mM. Importantly, this inhibition was accompanied by a significant decrease in extracellular lactate content. siRNA inhibition of MCT1 expression also showed a significant decrease in total cell biomass.

Conclusions: In this study, we evaluated the effect of MCT1 inhibition in cervical cancer cells and observed a significant decrease in total cell biomass which may be a result of inhibition of cell viability, cell proliferation and/or induction of apoptosis. Further studies are needed to better comprehend the mechanisms by which MCT inhibition exerts its effect on cervical cancer cells, however, by the results herein presented a promising therapeutic target can be anticipated for this type of tumours.

352 POSTER

Involvement of Grb2 adaptor protein in nucleophosmin-anaplastic lymphoma kinase (NPM-ALK) mediated signaling and anaplastic large cell lymphoma growth

L. Riera[1], E. Lasorsa[1], C. Ambrogio[1], N. Surrenti[1], C. Voena[1], R. Chiarle[1]. [1]*University of Torino, Biomedical Science and Human Oncology CeRMS, Torino, Italy*

Background: Most Anaplastic Large Cell Lymphoma (ALCL) express oncogenic fusion proteins derived from chromosomal translocations or inversions of the Anaplastic Lymphoma Kinase (ALK) gene. Frequently ALCL carry the t(2;5) translocation that fuses ALK gene to Nucleophosmin (NPM1) gene. NPM-ALK mediated transforming activity induces different pathways that control lymphoma cells proliferation and survival. Grb2 adaptor protein is thought to play an important role in ALK-mediated transformation, but its interaction with NPM-ALK and its functions in ALCL is still unclear.
In this work we focused on Grb2 binding to NPM-ALK, its phosphorylation by the fusion protein and its role in regulating signaling pathways and proliferation of ALCL cells.
Methods: Human embryonal kidney cells HEK-293T were transfected with different Grb2 and/or NPM-ALK constructs and immunoprecipitation and immunoblot analysis were performed. All Grb2 and all kinase mutants were generated by PCR-based mutagenesis. Inducible ALK and Grb2 shRNA SU-DHL-1 and TS cells were obtained by co-transduction with pLV-tTRKRAB (TTA) vector and pLVTHM vector containing the H1 promoter shRNA cassette. NPM-ALK or Grb2 silencing is achieved when 1μg/mL of doxycyclin is added to the medium for 72 hours. To generate Grb2 shRNA-resistant constructs, wild-type or Y160F Grb2 were mutated in 4 bases in the sequence corresponding to the shRNA (Grb2INT3/4). Co-culture and proliferation experiments were performed.
Results: In this study we demonstrate that Grb2 binds to active NPM-ALK and is phosphorylated in human ALCL cells. We identified Y160 as major phosphorylation site of Grb2 by NPM-ALK. We found that Y160 of Grb2 is phosphorylated also by other oncogenic fusion tyrosine kinases such as TPR-MET, BCR-ABL and TEL-JAK2, as well as by wild-type receptor tyrosine kinases, such as ALK and MET. Further, we show that NPM-ALK combined mutations in Y152–156, Y567 and P415–417 almost completely abrogated Grb2 binding and Y160 phosphorylation. Finally, shRNA knock-down experiments showed that Grb2 is essential for SHP2 activation in ALCL and is required for sustained ALCL cell growth.
Conclusions: Grb2 silencing in ALCL cells strongly impaired cell proliferation, suggesting that Grb2 is fundamental for the full activation of a signaling cascade that involves Shc and SHP2 and assures lymphoma cells proliferation. Thus, Grb2 could represent a potential target to control cell proliferation in NPM-ALK mediated lymphomas.

353 POSTER

Integrating alternative splicing studies as a tool for innovative therapeutical interventions: focus on novel drug targets and novel epitopes

A. Casagrande[1], M. Pando[1], L. Desire[1]. [1]*Exonhit Therapeutics, Therapeutic division, Paris, France*

Alternative RNA splicing is a key molecular mechanism for the generation of functional protein diversity. Abnormal alternative splicing can occur in cancer, resulting in the production of novel transcript variants or in an imbalance between mRNA isoforms. In both cases, it can affect the global pattern of protein expression within a cell, sustain tumour growth or affect drug response. Therefore, identification of cancer-associated alternative splicing variants may represent a significant step forward and potential source of new clinical diagnostic, prognostic and therapeutic strategies.
Results: ExonHit has generated discovery engines aimed at studying alternative splicing and is currently building libraries of alternative splicing events that are deregulated in cancer and in cases of therapy resistance. Here, using ExonHit's Genome Wide SpliceArray™ microarray, we investigated patterns of alternative splicing in diverse human cancers as well as in drug resistance models. Distinct splicing patterns were evidenced using principal component analysis and through statistical analysis of differential splicing. We show that a number of already targeted genes, as well as drug resistance genes, in fact undergo alternative splicing, which can ultimately affect drug response. We also implemented bioinformatic processes and selectivity filters that allowed to identify (1) alternatively splicing variants with altered druggable domains and (2) splicing variants that generate novel cell surface epitopes. Using this strategy, we show for example how the small GTPase Rac1 which is subjected to alternative splicing to generate the self-activated variant Rac1b, can be targeted through isoform-selective medicinal chemistry programs. In addition, we illustrate how such strategy can help to identify a number of other alternatively spliced transcripts containing novel amino acid sequences that can be used as novel epitopes. These novel epitopes-containing variants are now being used to target monoclonal antibodies for therapy.
Conclusion: Alternative RNA splicing offers a currently underexploited source of novel disease targets. Our results demonstrate a significant contribution of splicing to cancer development and drug response. Platforms dedicated to studying alternative splicing can be integrated into discovery processes to allow identification of novel targets for drug discovery which can then be subjected to innovative therapeutical interventions based on splicing variant-selective drug design, antisense- or antibody-based therapies.

354 POSTER

Investigations on organic anion-transporting polypeptides 1A2, 1B1 and 1B3 in colon cancer as potential targets for cancer therapy

V. Kounnis[1], E. Ioachim[2], M. Svoboda[3], I. Sainis[1], C. Ausch[4], G. Hamilton[5], T. Thalhammer[3], E. Briasoulis[1]. [1]*University of Ioannina, Cancer Biobank Center, Ioannina, Greece;* [2]*Hatzikosta General Hospital, Pathology Department, Ioannina, Greece;* [3]*Medical University of Vienna, Department of Pathophysiology and Allergy Research, Vienna, Austria;* [4]*Medical University of Vienna, Clinic for Surgery, Vienna, Austria;* [5]*LBI-Cluster for Translational Oncology, Vienna, Austria*

Background: Organic Anion-Transporting Polypeptides (OATP) selectively facilitate the uptake of endogenous substrates and drugs into cells. Previous studies suggest that certain OATPs are expressed in several cancers, which profiles them as potential target candidates for novel cancer therapeutics (I. Sainis *et al.*, *Mar Drugs* **8**, 629, 2010).
Materials and Methods: We investigated the expression of OATPs 1A2, 1B1, and 1B3 in human colon cancer. We studied their expression at the mRNA level by TaqMan real-time RT-PCR and at protein level by immunohistochemistry, in human colon cancer material and in four colon cancer cell lines (Caco, C205, HT29 and L174T). For immunohistochemistry we used the following antibodies: polyclonal rabbit SSV anti-OATP1A2, monoclonal mouse ESL anti-OATP1B1 and monoclonal mouse MDQ anti-OATP1B1/1B3.
Results: Using frozen samples from cancerous and adjacent non-cancerous colon tissues, we found OATP1B3 mRNA significantly expressed in 18/20 cancerous samples (where it reached 8.3-fold levels over control), as was also in the HT-29 and L174T cell line (4.7-fold enrichment). It normal colon OATP1B3 mRNA levels were undetected. Interestingly, OATP1A2 mRNA expression was also detected in the CaCo cell line, while HT29 expressed OATP1B1. The immunohistochemical study revealed that OATP1A2 and 1B1 were expressed in all studied cases while1B3 (by using the mMDQ antibody which also recognizes an epitope shared by 1B1) was expressed in 27/30 samples. Interestingly in positive cases, almost all cancer cells were stained positive. Furthermore, OATP1A2 protein expression was intense in 3/4 of the cases studied while 1B1 and 1B1/1B3 expression was weak in 40% and 63.3% respectively. Investigation of cancer-associated mutations of 1B3 is under way and will be presented.
Conclusions: Organic Anion-Transporting Polypeptides 1A1, 1B1 and 1B3 are differentially expressed in colon cancer. We suggest that further investigation of OATPs in colon and other cancers is warranted, in search of new cancer targets that may offer perspectives for the development of novel targeted cancer therapies.

355 POSTER

Identification of an inhibitor for melanoma cell migration through the inhibition of Pirin

I. Miyazaki[1], S. Simizu[1], H. Osada[1]. [1]*RIKEN, Chemical Biology, Saitama, Japan*

Background: Bcl3 was originally identified as a putative proto-oncogene which frequently rearranged in chronic B cell lymphocytic leukemia. Bcl3 interacts with NFkB by binding to p50 and p52. The overexpression of Bcl3 was reported to enhance cell survival, proliferation and tumor malignancy. Pirin is known to be bound to Bcl3, however, the exact roles of Pirin in tumor cells have not been clarified.
Materials and Methods: To discover the Pirin ligands, we have carried out a screening with the aid of a chemical array. In this screening system, small-molecules are covalently immobilized on the glass slides through a