to characterize in vivo the anti-vascular approach after PDT using the conjugated PS, compared to the non-conjugated PS.

The anti-vascular effect, for the conjugated PS, was characterized by a reduction in blood flow around 50% during treatment, a loss of the CD31 labelling integrity in endothelial cells from two hours post-PDT. In fine, 4 hours post-PDT, we observed for the conjugated-PS, microhemorrhages, vascular stasis and lumen trombosis confirmed by a decrease of the fibrinogen diffusion in tumor tissue. Following PDT, for this PS, endothelial cells became rounded but without change of morphological characteristics of the ultrastructures. In vivo, the photodynamic efficiency with the conjugated PS induced a statistically significant tumor growth delay compared to the non-coupled PS.

This targeting strategy of NRP-1 using a heptapeptide displays the potential of anti-vascular effect of PDT for glioblastomas treatment.

101 Poster

Differential role of choline kinase alpha and beta isoforms in human carcinogenesis

D. Gallego-Ortega[1], A. Ramirez de Molina[1], M.A. Ramos[1], F. Valdes-Mora[1], J. Sarmentero-Estrada[1], J.C. Lacal[1]
[1]Centro Nacional de Biotecnologia-CSIC, TCD-Pharma, Madrid, Spain

BACKGROUND: Overexpression of choline kinase alpha (ChoK alpha) has been related with cancer onset and progression. Two human genes, ChoK alpha and ChoK beta, code for enzymes with 65% homology and reported choline kinase activity. Here we compare the in vitro and in vivo characteristics of ChoK alpha1 and ChoK beta, and their potential role in carcinogenesis.

MATERIALS AND METHODS: in vitro choline kinase and ethanolamine kinase activity assays and the analysis of phosphocholine or phosphoethanolamine production in whole cells were carried using ^{14}C labelling of the proper substrates followed by a thin layer chromatography analysis. In order to compare the tumorigenic ability of each ChoK isoform, soft agar anchorage-independent growth and in vivo tumorigenic assays in athymic mice were carried out. ChoK alpha or beta mRNA levels in human tumor-derived cell lines were quantified by real-time reverse transcriptase PCR.

RESULTS: Both ChoK alpha1 and ChoK beta, showed choline and ethanolamine kinase activities in cell extracts assays. However they behave differentially when overexpressed in whole human embrionary kidney 293T cells, suggesting the involvement of each ChoK isoform in distinct biochemical pathways under in vivo conditions. In addition, while over-expression of ChoK alpha1 is fully oncogenic, ChoK beta over-expression is not sufficient to induce in vitro cell transformation nor in vivo tumor growth. Furthermore, ChoK beta is not a downstream target of Ras and Rho GTPases, as previously demonstrated for ChoK alpha1, which is activated by Ras and RhoA. We also investigated the mRNA levels of ChoK alpha and ChoK beta in a panel of breast cancer cells lines, finding a significant up-regulation of ChoK alpha mRNA levels in the tumoral cell lines but no changes were found in ChoK beta mRNA levels, compared with their normal counterpart. Finally, MN58b, a previously described potent inhibitor of ChoK alpha1 with in vivo antitumoral activity, shows more than 20-fold higher efficiency towards ChoK alpha1 than ChoK beta.

CONCLUSIONS: This study represents the first evidence of the distinct metabolic role of the alpha1 and beta isoforms of choline kinase, suggesting different physiological roles, and their implications in human carcinogenesis. These findings are very relevant for the screening of ChoK inhibitors, and for the design of novel anti-ChoK drugs with potential antitumoral use that should be focussed on ChoK alpha.

102 Poster

Design of mechanisms of selective prodrug activation in the bone marrow microenvironment of experimental and human multiple myeloma

D.J. Mincher[1], E. Van Valckenborgh[2], L. Young[1], A. Turnbull[1], A. Di Salvo[1], K. Vanderkerken[2]
[1]Napier University, School of Life Sciences, Edinburgh, United Kingdom; [2] Vrije Universiteit Brussel, Haematology and Immunology, Brussels, Belgium

Multiple myeloma (MM) is an incurable B-cell cancer characterized by monoclonal proliferation of tumour cells in the bone marrow. MM cells secrete MMP-9, a matrix metalloproteinase (MMP) implicated in tumour invasion, migration and angiogenesis. MMPs have conventionally been targeted with inhibitors but without success in the clinic. In an alternative approach, we have sought to subvert the proteolytic activity of overexpressed MMPs to activate prodrug substrates selectively in the tumour microenvironment. We have earlier reported [Van Valckenborgh et al (2005) Leukemia 19: 1628 - 1633] that prototype oligopeptide substrates of MMP-9 are activated by the bone marrow (BM) cells of the 5T33MM murine model of multiple myeloma (MM). Towards control of the mechanism of prodrug activation in the BM microenvironment, we now report the design (by controlling binding to the S1' position in the active site of MMP-9) of novel latently fluorescent prodrugs of cytotoxic and vascular disrupting agents and demonstrate that selective cellular activation translates from the murine model to human MM cells. Novel cytotoxic DNA-topoisomerase inhibitors incorporating the oligopeptide sequences: D-ala-ala-ala-leu-gly~nva-pro (EV1) and D-ala-ala-ala-leu-gly~ile (EV2) when modified at their N-terminus with FITC, afford prodrugs EV1-FITC and EV2-FITC, respectively, which are efficiently activated by MMP-9 rich BM cells or homogenates from the 5T33MM model to release the active agent (as shown by self-reporting fluorescence release and HPLC-MS in vitro metabolism data). EV2-FITC at 20μM in 5T33MM-diseased BM homogenate (500 μg protein/ml) was metabolized to the active agent (complete at 6h) whereas essentially no metabolism (at 24h) occurred in BM homogenate from naïve animals; nor was the prodrug degraded in homogenates from the non-tumour bearing organs, including liver, of the 5T33MM mice. Furthermore, EV1-FITC and EV2-FITC were incubated with human CD138+ (MMP-9-expressing) MM cells immunomagnetically isolated from BM samples of MM patients. Addition of either prodrug (20 μM) in the absence of MMP inhibitors resulted in a high release of fluorescence. The MMP-2/9 specific inhibitor peptide CTT (50μM) inhibited fluorescence release when the MM cells were pre-incubated (1h) whereas the control peptide STT and the serine proteinase inhibitor aprotinin had no effect upon fluorescence release, consistent with MMP-9 mediated prodrug activation. We further show that the optimized oligopeptide sequences can be used to conjugate experimental vascular disrupting agents that are similarly selectively activated in the BM microenvironment. The data indicates that exploiting endoproteolytic activity and tumour phenotype is feasible and that controlling mechanisms of selective prodrug activation in the BM microenvironment has the potential to improve the therapeutic index for MM patients.

103 Poster

Artesunate mediates growth inhibitory effects in human pancreatic cancer cells through modulation of multiple signalling pathways

M.M. Youns[1], T. Efferth[2], A. Bauer[1], J. Reichling[3], J. Hoheisel[1]
[1]Deutsches Krebsforschungszentrum, Functional Genome Analysis, Heidelberg, Germany; [2] Deutsches Krebsforschungszentrum, Pharmaceutical Biology, Heidelberg, Germany; [3] Heidelberg University, Biology, Heidelberg, Germany

Introduction: Pancreatic cancer is one of the most aggressive human malignancies, with an extremely poor prognosis. Systemic chemotherapy with gemcitabine is regarded as the standard chemotherapeutic approach. However, the median survival is still only around 6 months.

Artesunate, ART, a semisynthetic derivative of artemisinin, the active principle of Artemisia annua L., initially described as an anti-malarial drug, revealed remarkable antineoplastic activity against tumor cells. In the present study, we evaluated the effect of Artesunate on BxPc-3 and Miapaca-2 pancreatic cancer cell lines and the mechanisms by which Artesunate affects tumor growth of the 2 human pancreatic cancer cell lines.

Methods: BxPc-3 (moderately differentiated) and Miapaca-2 (poorly differentiated) pancreatic cancer cell lines were treated with varying concentrations of ART and the effect was monitored by MTS assay for evaluating of cell proliferation and by flow cytometry and detection of cytoplasmic histon-associated DNA fragments for apoptosis evaluation. In addition cDNA array contained 7000 genes is then fabricated and used as a tool to identify differentially expressed genes upon treatment with ART 20μM for 48 h. The microarray data were validated by positive correlation with quantitative real-time RT-PCR in a subset of genes from BxPc-3 cell line (GDF15, GAAD45a, COX-2, VEGF, PCNA, FOS and DDIT3); Moreover pathways associated with these expression changes were identified using the Ingenuity Pathway Analysis tool (IPA).

Results: ART induces growth arrest and apoptosis in BxPc-3 and Miapaca-2 pancreatic cancer cell lines in a time and dose dependant manner, and the effect was more prominent with the poorly differentiated Miapaca-2 cells. The expression analysis identified a common set of genes that were regulated by ART in the two pancreatic cell lines. Association of modulated genes with biological functional groups identified several pathways affected by ART including cell signalling, cell cycle, cell differentiation and apoptosis. In addition we identified GDF15, GAAD45a, PCNA, FOS and DDIT3 to be novel candidate genes involved in NS-398 mechanism of action on human pancreatic cancer cell lines.

Conclusion: The molecular mechanisms of ART-induced growth inhibition in human pancreatic cancer cells depend upon the differentiation stage of the cell lines examined. Moreover, we introduced GDF15, GAAD45a, PCNA, FOS and DDIT3 to be the novel molecular targets of ART and a new insight for possible combination therapy with chemotherapeutic agents to decrease their side effects and improve their efficiencies.