is known that inhibitors of HDACs (HDACi) induce cell cycle arrest and apoptosis of pancreatic cancer cells, the contribution of the individual HDACs to the carcinogenesis of pancreatic cancer is unknown so far.

Materials and methods: Expression of HDAC2 in pancreatic ductal adenocarcinomas was investigated using immunohistochemistry of tissue microarrays. Function of HDAC2 was analyzed using RNA interference in pancreatic cancer cell lines. Results were reproduced using the selective class I HDACi valproic acid. Proliferation and viability was measured using BrdU and MTT assays, respectively. Apoptosis was analyzed using Hoechst stains and PARP western blots.

Results: Immunohistochemistry and scoring reveals for the first time the overexpression of nuclear HDAC2, especially in undifferentiated pancreatic ductal adenocarcinomas. The knockdown of HDAC2 neither impaired proliferation nor reduced viability of pancreatic cancer cell lines. Instead we observed a sensitization towards death receptor (TRAIL)- and chemotherapy (etoposide)-induced apoptosis. In line, the selective class I HDACi valproic acid sensitizes pancreatic cancer cells towards death receptor (TRAIL)- and chemotherapy (etoposide)-induced apoptosis in a time- and dose-dependent fashion, without change of proliferation and viability.

Conclusions: Taken together, these data suggest a pivotal role of HDAC2 in regulating anti-apoptotic signaling and therapeutic resistance in pancreatic ductal adenocarcinoma. Therefore, targeting HDAC2 could be a promising future approach for the treatment of this dismal disease.

269 Poster

Kallikrein-related peptidase 6 overexpression promotes non-small cell lung cancer cell proliferation and is associated with poor patient outcome

N. Heuzé-Vourc'h[1], C. Planque[1], S. Guyetant[1], C. Coco[1], C. Blechet[1], C. Parent[1], B. Brillet[1], P. Reverdiau[1], M.L. Jourdan[2], Y. Courty[1]
[1]Inserm U618, Faculty of Medicine, Tours, France; [2] Inserm U921, Faculty of Medicine, Tours, France

Background: The human kallikrein-related peptidases (KLK) are a family of serine proteases that are often aberrantly expressed in common human malignancies and contribute to neoplastic progression through multifaceted roles.

Materials and Methods: We evaluated KLK6 expression in the tumoral and normal adjacent lung tissue of 56 patients with Non-Small Cell Lung Cancer (NSCLC) by real-time RT-PCR and immunohistochemistry. To determine the impact of KLK6 overexpression on the growth of lung cancer cells, we integrated the cDNA encoding the complete sequence of KLK6, through homolog recombination, in a NSCLC line (A549 Flp-In) and determined the growth rate of two independent clones. Progression of the KLK6- and parental cells inside the cell cycle was assessed by flow cytometry following synchronization of cells at the end of the G1 phase with starvation and hydroxyurea treatments. Key regulator proteins of the cell cycle were analyzed by Western blot in synchronized and unsynchronized cells.

Results: We found KLK6 transcript up-regulation in tumor tissues from patients with NSCLC and association of KLK6 status with low patient survival. KLK6 immunoreactivity was restricted to epithelial cells of normal bronchi and detected in most of cancer samples, in which KLK6 signal intensity correlated with well differentiated tumors. Ectopic expression of KLK6 dramatically enhanced NSCLC cell growth. Analysis of cell cycle progression revealed that promotion of cell growth caused by KLK6 results from an acceleration of cell cycle progression through G1/S transition, which was accompanied with a marked increase of cyclin E and repression of p21. In addition, expression of KLK6 in NSCLC cells was associated with an increase of c-Myc that is well-know to promote cell-cycle progression via regulation of cyclin D/E activation and down-regulation of p21.

Conclusion: In conclusion, ectopic expression of KLK6 facilitates cell cycle progression, certainly through alteration of c-Myc and downstream key regulators, and thus promotes cell proliferation. Moreover, KLK6 is overexpressed in NSCLC and associated with poor prognosis. Altogether, those findings suggest that KLK6 might play a central role in NSCLC development and progression.

270 Poster

Antiproliferative effect of GNRH-III and GNRH-II peptide derivatives on MCF-7, T47-d and HT-29 cells

I. Szabo[1], S. Bosze[1], M. Kovacs[2], B. Vincze[3], O. Csuka[3], F. Hudecz[4], G. Mezo[4]
[1]Research Group of Peptide Chemistry at Eötvös L. University Hungarian Academy, Eötvös L. University, Budapest, Hungary; [2] University of Pecs, Department of Anatomy, Pecs, Hungary; [3] National Institute of Oncology, Biochemical Department, Budapest, Hungary; [4] Research Group of Peptide Chemistry Hungarian Academy of Science, Eötvös L. University, Budapest, Hungary

The hypothalamic decapeptide gonadotropin-releasing hormone (GnRH-I; <EHWSYGLRPG-NH_2) is the central regulator of reproductive system through the stimulation of the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Some kinds of tumour cells (e.g. breast, prostate, colon) produce GnRH and express its receptors. GnRH-II (<EHWSHGWYPG-NH_2) is also expressed in humans and it has more inhibitory effect to tumour cell replication than GnRH-I. GnRH-III (<EHWSHDWKPG-NH_2) is a weak GnRH agonist. It has antiproliferative effect without significant endocrine effect therefore it could be used as a selective antitumour agent in the cancer therapy. We have found that symmetric dimmer derivatives of GnRH-III were more potent for the inhibition of tumour growth in vitro and in vivo, but their endocrine effect was even lower than the monomer had.

Our aim was to synthesise new asymmetric dimer derivatives of GnRH-I or GnRH-II and GnRH-III which increased receptor binding activity and can enhance the antiproliferative effect of GnRH-III (20%).

GnRH-derivatives were synthesized by solid phase synthesis using mixed Fmoc and Boc strategies. Different natural GnRH-derivatives were coupled to directly or through a spacer each other forming asymmetric dimers. The following dimers were produced in solution via thioeter linkage: GnRH-I-GnRH-III([VD-1]; [VD-12]; [VD-3]; [VD-31]) and GnRH-II-GnRH-III ([VD-2]; [VD-22]).

The receptor binding assay was used for determination of LH secretion of different GnRH derivatives on rat pituitary cells. We found that the asymmetric dimers retained the endocrine activity of natural GnRH-I or GnRH-II. The coupling of GnRH-III to the modified GnRH-I or GnRH-II did not disorder this effect.. Dimers VD-2 and VD-22 evoked LH release only in the higher concentration, but dimers VD-1 and VD-12 were more potent in the lower concentration. VD-31 dimer had the highest endocrin activity.

The in vitro cytostatic and antiproliferative effect of GnRH-derivatives were studied on MCF-7 and T-47D human breast cancer, HT-29, human colon carcinoma cell lines. We found that no cytostatic effect of the asymmetric dimers was observed on MCF-7 and HT-29 cell lines in the studied concentration range.

The asymmetric dimers had different antiproliferative effect on MCF-7, T-47D and HT-29.The most sensitive cell was T-47D for these asymmetric dimers. Dimer [VD-12] was the most potent (80%) on T-47D cells.

271 Poster

Identification of DUSP1/MKP1 mediated pathways in lung cancer progression

V. Moncho Amor[1], I. Ibanez de Caceres[1], E. Bandrés[2], J.L. Orgaz[3], I. Sánchez-Pérez[1], B. Jiménez Cuenca[3], J. Garcia-Foncillas[4], R. Perona[5]
[1]Instituto de Investigaciones Biomedicas, Experimental Models for Human Diseases Studies, Madrid, Spain; [2] Centro de Investigación Médica Aplicada, Linea de Farmacogenomica, Madrid, Spain; [3] Instituto de Investigaciones Biomedicas, Cellular Biology, Madrid, Spain; [4] Centro de Investigación Médica Aplicada, Linea de Farmacogenomica, Pamplona, Spain; [5] Instituto de Investigaciones Biomedicas Centro de Investigación Biomédica en Red de Enfermedades Raras, Experimental models for Human Diseases Studies, Madrid, Spain

Mitogen-activated protein kinase (MAPK) signalling pathways are activated in response to a diverse array of extracellular stimuli, cell proliferation, differentiation or transformation. MAPK and JNK/SAPK activation requires phosphorylation of both threonine and tyrosine residues, that are dephosphorylated by protein phosphatases, resulting in inactivation of MAP kinases. The enzymes involved in this inactivation are the dual specific family of protein phosphatases (DUSP). DUSP1 was the first one DUSP to be identified and is encoded by an immediate early gene that has been shown to be stimulated under conditions of inflammation and stress, oxidative stress or growth factors. In addition, we have shown previously that DUSP1inhibition decreases tumor growth and sensitizes cancer cells to conventional chemotherapy, resulting in a NSCLC tumor size arrest.

The aim of this work is to gain insight into the cellular pathways involving DUSP-1 actions by using a double strategy that combines siRNA and microarray technologies. This strategy will provide a differential expression profile of genes regarding functionality of DUSP1.

The present study is based on a comparative analysis of RNA expression of the NSCLC H460 and H460-siDUSP1 cell lines. Total RNA from both cell lines was extracted, reverse-transcribed and hybridized into an array platform containing the whole human genome (affymetrix Human Genome U133 Plus 2.0). After data normalization, we selected 136 genes at least 3-fold up and down regulated comparing the interfered versus wild type cell lines (H460 and H460-siDUSP1). Posterior gene ontology analysis identified some of specific biological pathways related to angiogenesis, MAP kinase phosphatase activity, cell-cell signalling and growth factor activity. We validated the gene expression by real time PCR and pathways obtained by the gene ontology study were confirmed by the next complementary assays:

To study the effect of DUSP-1 in angiogenesis, movement of human umbilical endothelial cells (HUVEC) was assessed using Boyden chamber assay. In this assay, HUVEC cells showed an increased migration when media was obtained from H460 rather than H460-siDUSP1. The effect of DUSP-1 in migration was tested by the wounding healing technique. In this case, only H460 cells closed up the wound at 24 hours of post-wounding. H460-siDUSP1 showed lower invasivity potential through the matrigel than H460 cell line. To analyze the role of DUSP-1 in tumorogenesis, 16 nu_/nu_ mice were inoculated with H460 or H460-siDUSP1 cell lines. The last one induced less number of tumours with a slower growth rate than H460 wt.

All together these results indicate that the interference of DUSP1 in H460 cells reduce angiogenesis, cell migration, invasivity, and tumorogenicity, suggesting a main role of DUSP-1 in lung cancer progression.

272 Poster

Telomere function and p16/RB and p53-mediated senescence pathways in human cancer

T. Fernández-Marcelo[1], C. Frías[1], C. De Juan[1], A. Morán[1], P. Ortega[1], J.A. López-Asenjo[2], A. Sánchez-Pernaute[3], A.J. Torres[3], M. Benito[1], P. Iniesta[1]
[1]Universidad Complutense de Madrid, Bioquímica y Biología Molecular II, Madrid, Spain; [2] Hospital Clínico San Carlos, Servicio de Anatomía Patológica, Madrid, Spain; [3] Hospital Clínico San Carlos, Servicio de Cirugía II, Madrid, Spain

According to telomere hypothesis, telomere shortening prevents somatic cells from dividing and status senescence. However, cell may escape from the senescence barrier if key tumour suppressor genes, especially p53 and/or p16/RB lose their function. Previous results from our group in colorectal cancer (CRC) and non-small cell lung cancer (NSCLC) indicated a differential clinical implication for combined telomerase activity and p16 expression analyses. In this work, our main aim consists of evaluating telomere function in relation to p16/RB and p53-mediated senescence mechanisms in different types of human tumours.

We analyzed telomere function by evaluating telomerase activity and telomere length in a series of CRC and NSCLC tumours obtained from patients who suffered potentially curative surgery. p16/RB and p53-mediated senescence pathways were investigated performing expression assays with oligonucleotide arrays containing 113 genes related to each one of the two senescence pathways. Also, prognosis studies were established.

In NSCLC our data indicated a protective effect for p16 expression in patients showing tumours with significant telomere attrition ($P < 0.05$). However, in CRC it seems more relevant the effect of p53-mediated senescence pathway. Thus, p53 positive expression was a protective parameter in patients with tumours underlying alterations in telomere function. In order to better investigate different roles of these senescence pathways in CRC and NSCLC, following we performed expression studies by arrays. As result, a number of genes from the two pathways showed different expression profiles in relation to telomerase activity and/or telomere length in the two tumour populations considered in this work.

In conclusion, our results suggest a differential impact for p16/RB and p53-mediated senescence pathways in CRC and NSCLC, in relation to telomere function.

273 Poster

The vasoactive intestinal peptide-receptor system is involved in human glioblastoma cell migration

S. Cochaud[1], A.C. Meunier[1], L. Chevrier[1], J.M. Muller[1], C. Chadéneau[1]
[1]CNRS UMR 6187, Physiopathologie de la Communication et de la Différenciation Cellulaires, Poitiers, France

Background: Glioblastoma multiforme (GBM) is the most aggressive form of primary brain tumor in adults. This cancer has a highly proliferative and invasive nature and is characterized by inter- and intratumoral heterogeneity. The neuropeptides of the vasoactive intestinal peptide (VIP) family and their receptors (VIP-receptor system) play an important role in the regulation of normal neuronal development, in growth rate of numerous cancer cell lines and also in migration in prostatic and colonic cancer cell lines. Little is known about the involvement of this system in proliferation or migration of GBM cells. Materials and methods: Expression of the VIP-receptor system was studied by RT-PCR, western immunoblotting and binding experiments in two human glioblastoma cell lines, M059K and M059J, established from different regions of a same tumor. The effects of neuropeptides or receptor antagonists of the VIP-system on proliferation or migration of these cells were tested by MTS proliferation and wound healing assays, respectively. The rearrangement of the actin cytoskeleton was visualized by immunofluorescence. Results: The VIP-receptor system was less expressed in M059J cells than in M059K cells. Compared to M059K cells, M059J cells expressed only 20% of VPAC1 receptors, one of the receptors of the system. No effect on proliferation was observed in both cell lines, but differences in migration were found. M059J cells which express less the VIP-receptor system than M059K cells migrated faster. Migration was decreased in neuropeptide-treated M059J cells and was increased in VPAC1 receptor antagonist-treated M059K cells. In agreement with stimulation of migration, a reorganization of the actin cytoskeleton in filopodia was observed in the M059K cells treated with antagonists. Conclusions: The VIP-receptor system is expressed differentially in M059J and M059K cell lines, reflecting intratumoral heterogeneity, and is involved in migration of these cells.

274 Poster

Marrow-derived mesenchymal stem cells (MSCs) stimulate breast cancer cell secretion and expression of chemokines

F.T. Martin[1], R.M. Dwyer[1], J.M. Murphy[2], F.P. Barry[2], T. O'Brien[2], M.J. Kerin[1]
[1]National University of Ireland Galway, Surgery, Galway, Ireland; [2] National University of Ireland Galway, Regenerative Medicine Institute, Galway, Ireland

Background: Breast cancer related bone metastasis remains a devastating progression of disease for which no curative therapy exists. Mesenchymal Stem Cells (MSCs) within the bone marrow stroma are postulated to play a role in developing a pre-metastatic niche to support the engraftment and progression of disseminating breast cancer cells. The potential role of MSCs in tumorigenesis is thought to be as a result of their ability to secrete a range of chemokines. The aim of this study was to investigate the effect of direct co-culture with MSCs on breast cancer cell chemokine secretion and gene expression.

Materials and Methods: MSCs were isolated from bone marrow aspirates of healthy volunteers, and their ability to differentiate along connective tissue lineages confirmed. Breast Cancer Cell lines, MDA-MB-231 and T47D, were cultured individually and also on a confluent monolayer of MSCs. Conditioned medium was harvested at 48 and 72 hours from cells cultured individually or in co-culture, and the concentration of chemokines, Stromal Cell-Derived Factor-1α (SDF-1α) and Monocyte Chemotactic Protein-1 (MCP-1), were quantified by ELISA. Epithelial cell specific beads were used to retrieve breast cancer cells following co-culture with MSCs, for RNA extraction. Expression of MCP-1 and SDF-1α was quantified in retrieved tumour cells by RQ-PCR.

Results: Breast cancer cells cultured alone secreted low levels of MCP-1 (48 ± 21pg/ml - Mean ± SEM) while MSCs secreted relatively high levels (1266±141pg/ml). Following 72hrs co-culture, a synergistic effect was observed with MCP-1 levels significantly higher than those seen in the individual populations (MDA-MB-231 + MSC: 7175 ± 1732 pg/ml $p<0.05$, T47D + MSC: 4853 ± 1295 pg/ml, $p<0.05$). In contrast, following 72hrs in co-culture conditions, there was a net decrease in SDF-1α detected, compared to levels secreted by the individual populations (range 27%- 63% decrease in SDF-1α).RQ-PCR analysis of RNA from breast cancer cells retrieved following co-culture with MSCs revealed upregulation of MCP-1 expression in both breast cancer populations, while SDF-1α expression remained virtually unchanged.

Conclusion: MSCs alter the secretion and expression of MCP-1 and SDF-1α in breast cancer cells following direct co-culture. Considering the potential role of these chemokines in developing and cultivating the tumour microenvironment, these interactions may play an important role in the development of bone metastases.

275 Poster

A potential role for vascular endothelial growth factor-D as an autocrine factor for human gastric carcinoma cells

M. Tanaka[1], Y. Kitadai[1], M. Kodama[1], K. Shinagawa[1], T. Sumida[1], T. Kuroda[1], S. Tanaka[2], K. Chayama[1]
[1]Hiroshima University, Department of Medicine and Molecular Science, Hiroshima, Japan; [2] Hiroshima University Hospital, Department of Endoscopy, Hiroshima, Japan

Background: Vascular endothelial growth factor (VEGF)-D induces lymphangiogenesis by activating VEGF receptor (VEGFR)-3, which is expressed mainly by lymphatic endothelial cells. VEGFR-3 has also been detected in several types of malignant cells, including lung, colorectal, and prostate carcinoma cells, but the significance of VEGFR-3 expression by malignant cells remains unclear. We have reported the expression and role of the VEGF-C/VEGFR-3 axis in human gastric carcinoma, but a role of VEGF-D in gastric carcinoma has not been characterized. In this study, we examined the expression and function of VEGF-D/VEGFR-3 in human gastric carcinoma cells.

Materials and Methods: We examined the expression of VEGF-D and VEGFR-3 in four human gastric carcinoma cell lines by reverse transcription-polymerase chain reaction. We also used cDNA microarrays