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

to examine the effect of VEGF-D on the expression of genes associated with disease progression in VEGFR-3-expressing KKLS cells. To stimulate VEGF-D/VEGFR-3 autocrine signaling, a VEGF-D expression vector was transfected into KKLS cells, and stable transfectants were established. VEGF-D-transfected cells and control cells were then transplanted into the gastric wall of nude mice (i.e., orthotopically).

Results: Gastric carcinoma cell lines constitutively expressed VEGF-D mRNA. Two of the four cell lines expressed VEGFR-3 mRNA and protein. In vitro treatment of KKLS cells with exogenous VEGF-D stimulated cell proliferation and increased expression of mRNAs encoding Bcl2 and autocrine motility factor receptor. Proliferation of VEGF-D-transfected cells transplanted into the gastric wall of nude mice was greatly increased compared to that of control cells. VEGF-D transfection into KKLS cells resulted in inhibition of apoptosis and stimulation of angiogenesis and cell proliferation. However, lymphangiogenesis was not increased in response to VEGF-D transfection.

Conclusions: Human gastric carcinoma cell lines express VEGF-D and VEGFR-3. VEGF-D may be involved in the progression of human gastric carcinoma by acting via autocrine and paracrine mechanisms.

276 Poster

Monitoring of tumor progression using bioluminescence imaging in a nude mice orthotopic model of human small cell lung cancer

S. Iochmann[1], S. Lerondel[2], C. Bléchet[1], S. Pesnel[2], N. Heuzé-Vourc'h[1], Y. Gruel[1], A. Le Pape[2], P. Reverdiau[1]
[1]Inserm U618, Team 3, Tours, France; [2] Cdta-cnrs, Cipa, Orléans, France

Background: Lung cancer is the main cause of cancer deaths throughout the world and a clinically relevant animal model of human small cell lung carcinoma (SCLC) should be useful to study the molecular aspects of the tumor progression and test the efficiency of new therapeutic agents. In this study, we generated a reproductible and reliable nude mice orthotopic model of human SCLC based on NCI-H209 tumor cells genetically modified to express firefly luciferase. Materials and Methods: NCI-H209 cells were transfected with pCMV-luc plasmid and clones highly expressing luciferase were isolated and amplified. Cells were analyzed for long-term bioluminescent stability and a clone was subcutaneously passaged twice in vivo to enhance tumorigenicity. Cells resuspended in Matrigel® and/or EDTA RPMI medium containing a Tc99M colloid were implanted intrabronchially using a catheter inserted into the trachea and positioned into the right main bronchus using interventional imaging. Punctual deposition of cells was then assessed by scintigraphy. Results: Only tumor nodules were observed into lung and trachea when cells were implanted with EDTA. Lung tumor invading parenchyma were present in 40% of the mice with Matrigel® and improved to 75% with EDTA and Matrigel®. The growth of the primary tumor was sensitively and non-invasively followed and quantified by bioluminescence imaging using a CCD-camera. This tool allows a real-time monitoring of tumor progression on the same animals over a 2-12 week period. Combination of 3D bioluminescence imaging and computed tomography scanning was used to further document tumor location and measurement. Subsequently, the histological analysis of tissue sections confirmed the presence of a lung tumor. Conclusion: Our nude mice orthotopic model resembles various stages human small cell lung carcinoma, and then could be useful for evaluating new therapeutic strategies.

277 Poster

Voltage-gated sodium channels activity promotes cysteine cathepsins-dependent invasiveness of human cancer cell lines

L. Gillet[1], S. Roger[1], P. Besson[1], J. Gore[1], P. Bougnoux[1], F. Lecaille[2], G. Lalmanach[2], J.Y. Le Guennec[1]
[1]INSERM U921, Université François-Rabelais, Tours, France; [2] INSERM U618, Université François-Rabelais, Tours, France

Background: Various molecular isoforms of voltage-gated sodium channels (Na_v) are functionally expressed in several cancer types of non-excitable, epithelial tissues (breast, prostate, lung). In the aggressive breast cancer cell line, MDA-MB-231, we found that the $Na_v1.5$ isoform is involved in cell invasiveness. Our goal is to understand the link between Na_v activity and extracellular matrix proteolysis.

Materials and methods: To study the activity of the protein, we used the patch clamp technique in the whole cell configuration. RT-PCR and western-blot were used to identify the isoforms of the different proteins studied. The regulation of genes transcription was studied by quantitative PCR. Enzymes activities were determined using fluorogenic peptidyl substrates. Intracellular pH was monitored using the ratiometric fluorescent dye BCECF and cell surface pH using fluorescein-conjugated DHPE (N-(fluorescein-5-thiocarbamoyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine).

Results: The activity of Na_v promoted the invasive properties of cancer cells. The inhibition of Na_v by the specific blocker tetrodotoxin (TTX) impaired the invasivity of MDA-MB-231 cells. These cells express different functional cysteine cathepsins. Matrigel™ invasion was decreased by 65% in presence of a broad spectrum, membrane-impermeant inhibitor of cathepsins, and was specifically decreased by inhibitors of cathepsins B and S (CA-074 and Z-FL-COCHO). The association of these inhibitors with TTX demonstrated no further effect, indicating the regulation of extracellular cathepsins activity by Na_v. Blockade of $Na_v1.5$ activity by TTX for 24h had no effect on the transcription of genes encoding for cathepsins, cystatins (cathepsins endogenous inhibitors) or $Na_v1.5$. Likewise, no difference in the amount of cysteine proteases released in the extracellular medium was observed by western blot or by enzymatic titration assay, indicating that Na_v does not influence secretion and membrane-associated cathepsins activity. Conversely, we found that Na_v activity leads to an intracellular alcalinization and thus participates in the acidification of the pericellular space. Such an acidification is favourable to the activity of cysteine cathepsins.

Conclusion: This work suggests that Na_v activity facilitates invasiveness of cancer cells by promoting pH-dependent activation of cysteine cathepsins.

278 Poster

Expression of the carboxy-terminal tail of connexine 43 could induce similar effects to full-length connexin 43 on tumor proliferation

S.M. Crespin[1], W.C. Sin[2], J.F. Bechberger[2], M. Mesnil[1], C.C. Naus[2]
[1]University of Poitiers IPBC UMR CNRS 6187, Department of Physiopathology of Cellular Differentiation and Communication, Poitiers, France; [2] University of British Columbia, Department of Cellular and Physiological Sciences, Vancouver, Canada

Background: Accumulating data seem to associate connexin 43 (Cx43), a structural protein of Gap Junction Intercellular Communication (GJIC) and tumorigenicity. The aim of our study was to establish the relative importance of GJIC versus the intracellular signaling pathways mediated by the carboxyl tail (C-tail).

Materials and methods: LN18 human glioma cell line expressing low levels of endogenous Cx43 was transduced by retroviral infection with different forms of Cx43: (1) the full-length Cx43, (2) a truncated TrCx43 lacking the C-tail, and (3) the carboxyl tail only, 243-Cx43. Proliferation rate was determined by cell counting, migration behavior was studied by wound healing assay and transwell assay; and oncogenicity was examined by anchorage-independence soft agar assay.

Results: As expected, Cx43 was localized to the cell membrane in LN18-Cx43 cells. LN18 TrCx43 showed a membranous and cytosolic Cx43 staining and LN18 243-Cx43 exhibited a diffuse signal. Increased number of coupled cells (28±4 coupled cells versus 10±2 for LN18 mock) was detected only in LN18-Cx43 but not LN18-TrCx43, indicating that the C-terminal tail of Cx43 is needed for optimal GJIC. There is no significant difference in growth rate between these three lines on monolayer cultures. Interestingly, expression of all three Cx43 constructs reduced the oncogenicity of LN18 cells – they had a lower number of colonies and smaller colonies than LN18-mock in soft agar. Un-expectably, all three Cx43 constructs were equally well in increasing migration rate in wound healing assays (the transfected LN18 lines expressing different Cx43 constructs moved over 200 μm in 24 hours versus 150 μm for the LN18 mock).

Conclusions: Taken together, these results indicate that 1) GJIC is not required for Cx43-dependent cell motility, 2) the similarities in cellular effects observed with TrCx43 and 243-Cx43 suggest that Cx43 affect cell motility and growth by affecting two independent pathways. There is already evidence to suggest the extracellular domain of Cx43 is important for adhesion (Elias et al, 2007). Although our results indicate that a role of C-tail in modulating GJIC, a more important function of the C-tail of Cx43 in modulating tumorigenicity appear to be acting as a scaffold, bringing many signaling molecules together in close proximity.

279 Poster

Host cell recruitment by gliomas

J. Najbauer[1], M.E. Barish[2], E. Garcia[1], M.Z. Metz[1], M. Gutova[1], R.T. Frank[1], S.E. Kendall[3], C.A. Glackin[3], R. Bjerkvig[4], K.S. Aboody[1]
[1]Beckman Research Institute of the City of Hope, Hematology/Hematopoietic Cell Transplantation, Duarte, USA; [2] Beckman Research Institute of the City of Hope, Neuroscience, Duarte, USA; [3] Beckman Research Institute of the City of Hope, Molecular Medicine, Duarte, USA; [4] NorLux Neuro-Oncology, Biomedicine, Bergen, Norway

Malignant gliomas are the most common primary brain tumors and are considered among the deadliest of human cancers. Molecular, cellular and genetic analysis has advanced our understanding of these tumors, but little is known about the responses of the host brain and other organs to gliomas. Data suggest a two-way cell trafficking between tumor and host;