glioma cells spread into the normal brain parenchyma, whereas numerous cell types are recruited to the tumor.

We studied orthotopic implants of human glioma cell lines (U251, U87, D566) in nu/nu mice and human primary glioma in nu/nu rats. Glioma implants attracted large numbers of host nestin+ cells. Double-labeling for human and mouse nestin revealed an interdigitated network of tumor and host cells in glioma mass and at invasive tumor edge. PCNA immunostaning revealed areas of proliferative activity within glioma, as well as in ipsilateral SVZ. GFAP+ cells formed a distal halo around the primary tumor site, whereas Ms nestin+ cells surrounded the glioma proximally and penetrated into the tumor mass. The glioma mass displayed high levels of angiogenesis. Invasive glioma micro-foci (< 100 micrometers) contained Ms nestin+ cells with arbor-rich morphology, but these small tumor foci lacked blood vessels. SMA+ cells, which are likely vascular mural cells (pericytes or smooth muscle cells) were present within primary glioma mass and surrounding tumor foci. Ms nestin+ cells were also present at sites of vessel sprouting and bifurcation, suggesting a role of these cells in vessel formation. Close physical contact was apparent between Ms nestin+ and SMA+ cells during glioma neovascularization, a phenomenon known to be associated with TGF-beta signaling and endothelial cell-directed differentiation of mesenchymal cells into mural cells. In intracranial implants of primary human glioma in nu/nu rats, we detected distinct tumor phenotypes at each passage. Highly invasive, non-angiogenic tumor was associated with low passage number (1st), and less invasive, highly angiogenic phenotype was associated with higher (5th) passage number. In the latter, we observed Rat nestin+, glomerulus-like blood vessels, which recapitulated the morphology of malformed vasculature observed in patients with high-grade glioma. SDF-1 and its receptor CXCR4 were highly expressed in and around glioma, which may be involved in both tumor invasion and attraction of host cells. SDF-1 was also expressed on tumor-associated blood vessels, where it may serve as 'trap' for circulating cells, including cells derived from the bone marrow.

Our data suggest that glioma and host brain are connected by an intricate network, which includes recruitment of host cells to the tumor. Defining the role of recruited cells in the biology of gliomas may aid the development of novel anti-glioma therapies.

280 Poster

The role of translationally controlled tumour protein in tumourigenesis

H.J. Newbery[1], M. Brueser[1], I. Phillips[1], C.M. Abbott[1]
[1]University of Edinburgh, Medical Genetics Section, Edinburgh, United Kingdom

Background: Translationally controlled tumour protein (TCTP) is a highly conserved protein with numerous functions, including a role in cancer. It has been shown to interact with, and inactivate, the translation factor eEF1A (eukaryotic elongation factor 1A). However, it is unclear whether it binds to eEF1A1, the commonly expressed form of this protein, alone, or whether it also binds to eEF1A2, which has a more restricted expression pattern. The inappropriate expression of eEF1A2 in tissues in which it is not normally expressed is associated with cancer. If TCTP binds to eEF1A1 only, and not eEF1A2, then this would provide a possible explanation for the oncogenicity of eEF1A2.

Materials and Methods: Co-immunoprecipitation was used to pull down TCTP, and also any proteins with which it interacts. Antibodies specific for eEF1A1 and eEF1A2 were then used to determine to which translation factor TCTP binds.

Immunohistochemistry was also used on tissue microarrays, to determine whether TCTP is upregulated in cancer.

We have also used short interfering RNAs (siRNA) to knock down the expression of TCTP in different cell lines.

Results: 1. TCTP binds to both eEF1A1 and eEF1A2. 2. TCTP is upregulated in a high proportion of tumours. 3. We have successfully knocked down TCTP in different cell lines.

Conclusions: As TCTP binds to both eEF1A1 and eEF1A2, it is unlikely that the reason eEF1A2 is upregulated in cancer is due to its inactivation by TCTP. Additionally, we have confirmed a role for TCTP in cancer, as it is upregulated in tumour samples compared with normal tissues. Ongoing experiments will determine the effect on cellular proliferation and cell architecture when TCTP is knocked down.

281 Poster

Positional cloning of t(3;6) in rat endometrial cancer

E. Samuelson[1], S. Karlsson[2], A. Behboudi[2]
[1]University of Gothenburg, Cell and Molecular Biology-Genetics, Göteborg, Sweden; [2] University of Gothenburg, Clinical Genetics SU/Sahlgrenska Hospital, Göteborg, Sweden

Cytogenetic aberration and chromosomal rearrangement are common features of tumors. The consequence of balanced rearrangements can be deregulation of genes in the breakpoints or formation of a new gene, a fusion gene. Fusion genes may provide favorable properties to the tumors thereby paving the way to full malignancy. Expression of fusion genes is of decisive importance for diagnosis, classification, prediction of clinical outcome, and choice of therapy. Cytogenetic analysis and Spectral Karyotyping (SKY) analyses of 23 endometrial adenocarcionoma (EAC) tumors developed in females from BDII rat strain derived crosses illustrated that translocations involving rat chromosome (RNO) 6 were common among these tumors. Two tumors showed a similar translocation between RNO3 and RNO6, t(3;6) and a third tumor showed a complex form of translocation t(3;6) with a ladder like pattern in form of exchange of multiple chromosomal segments between these two chromosomes. In addition, yet three other tumors displayed translocations involving RNO6 fused with RNO10 or RNO16. Using Fluorescence in situ Hybridization (FISH) on metaphase spreads from these six tumors and DNA from BAC (bacterial artificial chromosome) clones as probes, positions of the chromosomal breaks in translocation events were determined. In dual-color FISH, we could successfully show that t(3;6) breakpoints in RNO3 and RNO6 were identical in two tumors, NUT97 and NUT98. In addition, FISH analysis revealed that RNO6 breakpoints in the other four tumors were not similar to that observed in NUT97 and NUT98, but derive from approximately the same region at the distal part of RNO6. This part of RNO6 is homologous to distal human chromosome 14q, which has been reported to be involved in balanced chromosomal aberrations in adenocarcionoma tumors in the ovary. These results may provide new insights into pathways involved in endometrial carcinogenesis.

282 Poster

The involvement of wnt beta-catenin signal pathway in the invasion and the migration of oral squamous cell carcinoma cells

S. Iwai[1], A. Yonekawa[1], C. Kong[1], K. Aota[1], M. Nakazawa[1], Y. Yura[1]
[1]Osaka University, Oral and Maxillofacial Surgery II, Osaka, Japan

Background and purpose: The Wnt signal pathway is involved in the carcinogenesis of various tumors including oral squamous cell carcinoma (SCC). In the presence of Wnt signals, Wnt receptors, which are frizzled homologs, activate the phosphoprotein Dishevelled and the ability of GSK-3beta to phosphorylate beta-catenin is then inhibited. Unphosphorylated beta-catenin is stable and accumulates in the cytosol and nucleus. In the nucleus, beta-catenin binds to T-cell factor (TCF)/lymphocyte enhancer-binding factor (LEF) to form a functional transcription factor which mediates the transactivation of target genes such as c-myc, cyclin D1, c-jun, fra-1, and u-PAR. In this results, invasiveness and migration increase in many kinds of tumor cells. We have reported the cytoplasmic and nucleous accumulation of beta-catenin in oral SCC. Then, we investigated the influence of the cytoplasmic and nuclear accumulation of beta-catenin on the oral SCC cell. Materials and methods: Oral SCC cell line were used in which beta-catenin expressed in the membrane but not in the cytoplasm and nuslei. Wild type beta-catenin cDNA containing the entire cording region and a mutated form of beta-catenin cDNA lacking exon3 including specific GSK-3beta phosphorylation sites were cloned into pUHD10-3 vector under regulation of a tetracycline-responsive promoter.These cDNAs was each cotransfected with pUHD15-1Neo, and stable cell lines were established. Results; Immunohistochemical staining using anti-beta-catenin antibody confirmed accumulation of beta-catenin in cytoplasm and nuclei of transfectants. In invasion assay and migration assay, invasion and migration activity of transfectants much more increased than those of parental cell line.Then, the transcriptional activity of Tcf DNA binding sequence in transfectants more increased than those of parental cell line. Then doxycycline reduced this activity. MMP-7 expression level of mRNA and activity of transfectants more increased than those of parental cell line. Further, Rearrangement of cytoskeleton protein and increase of activity of Rho family were observed in transfectants.

Conclusions; We suggested that in oral SCC cytoplasmic and nuclear accumulation of beta-catenin induced the increase of invasion and migration activity partially interacted with Tcf/lef transcriptional activity and partially through the rearrangement of cytoskeletal proteins and the activation of Rho family. Therefore, the malignancy of oral SCC increased interacting with wnt beta-catenin signaling pathway.

283 Poster

Modulation of cellular response to stable RNA silencing of tissue factor pathway inhibitor-2 in lung cancer cells

G. Gaud[1], S. Iochmann[1], B. Brillet[1], S. Petiot[1], C. Blechet[1], N. Heuze Vourc'h[1], Y. Gruel[1], P. Reverdiau[1]
[1]Inserm U618, IFR135, Tours, France

Introduction: Lung cancer is frequently diagnosed at an advanced stage and the malignant potential of this cancer depends on the ability of tumor cells to invade the surrounding tissue and form metastasis. This invasion

process involves ECM-degrading proteases and particularly matrix metalloproteinases (MMPs) that have been shown to be highly expressed and activated by serine proteases in tumoral microenvironment. By inhibiting plasmin, Tissue Factor Pathway Inhibitor-2 (TFPI-2) could modulate indirectly the activation of MMPs thus regulating ECM degradation and tumoral cell invasion. In the present study, we used a RNA interference method to stably knock down the expression of TFPI-2 in NCI-H460 non-small cell lung cancer cell line.

Methods: Micro interfering RNAs (miRNAs) were used to trigger sequence-specific TFPI-2 RNA degradation and then gene silencing. Two miRNA targeted TFPI-2 mRNA were designed and a non-silencing miRNA, showing no known homology to mammalian genes, was used as negative control. TFPI-2 mRNA level was measured by real-time RT-PCR and protein inhibition evaluated by western blot. Cell proliferation was measured by MTT assay. Cell migration was studied using a model based on Boyden chamber and using transwell inserts. To study the cell invasion through basement membrane components, cell culture inserts were coated with a thin layer of Matrigelâ.

Results: A specific inhibition of both TFPI-2 mRNA (between 76 and 85%) and protein was observed in NCI-H460 clones expressing miRNA. Cell proliferation was not modified by TFPI-2 RNA degradation. However, we showed that the downregulation of TFPI-2 expression was associated with a strong increase of cell invasion through basement membrane components while migration was less affected. Adhesion assays showed a slight effect of TFPI-2 inhibition on cell adherence to laminin and collagen IV matrix. Furthermore, TFPI-2 downregulation is associated with an increased expression of MMP-1 transcripts.

Conclusion: This study demonstrated that downregulation of TFPI-2 RNA by miRNA might favor the invasive behaviour of tumoral cell. To investigate these results, metalloprotease expression by tumoral cells will be now evaluated particularly when cocultured with stromal fibroblasts.

284 Poster

Tumor-stromal cell interactions modulate metalloproteinase and kallicrein expression in direct and indirect co-culture cell models

B. Brillet[1], S. Petiot[1], S. Iochmann[1], G. Gaud[1], C. Planque[1], C. Blechet[1], N. Heuzé-Vourc'h[1], Y. Gruel[1], Y. Courty[1], P. Reverdiau[1]
[1]Université François-Rabelais, Inserm U618, Tours, France

Background: The crosstalk between tumor cells and surrounding stromal fibroblasts is now considered crucial for cancer progression, particularly in invasive tumors such as lung carcinomas. Tumor-stromal cell interactions might provide signal for regulating protease and protease inhibitor secretion in the tumoral microenvironment and modulate extracellular matrix (ECM) proteolysis and then tumor invasion. The aim of this study was to develop co-culture models with cancer cells, derived from a non-small cell lung carcinoma (NSCLC), and fibroblast cells. Expression of several MMPs, kallicrein 6 and 8 (KLK) and Tissue Factor Pathway inhibitor 2 (TFPI-2) was then measured in these models.

Material and Methods: Two in vitro co-culture models were developed to evaluate the effects of direct or indirect contact between NSCLC NCI-H460 cells and CCD19-Lu fibroblast cells. In direct co-culture, both cells (ratio 1 :1) were cultured for 24 h in serum free medium. In indirect co-culture, conditioned media were collected from either confluent tumoral cells or fibroblasts grown in serum free medium during 24 h. Transcript levels of MMP-1, -2, -3, -9, -13 and –14, EMMPRIN (Extracellular Matrix MetalloPRoteinase Inducer), KLK6, KLK8 and TFPI-2 were measured using specific quantitative real-time RT-PCR. Protein expressions were evaluated by Western Blotting and immunofluorescence staining.

Results: We found a 3-fold and 8-fold increase of MMP-3 and MMP-9 expression respectively in the direct co-culture compared to cells grown alone. Although the level was lower, KLK6 mRNA was also enhanced in direct co-culture. In indirect co-culture with CCD19Lu cultured with NCI-H460 conditioned medium, we observed an increase in MMP-1, -3, -9 and TFPI-2 transcripts. Except for MMP3 and KLK6, no difference in transcripts level were observed in the other indirect co-culture model, i.e NCI-H460 grown in CCD19Lu conditioned medium.

Conclusion: Our results indicate that direct or indirect contacts between tumors and surrounding fibroblasts modulate the expression of various proteinases. This effect might be mediated by soluble or/and cell surface factors. Further investigations will be required to identify them.

285 Poster

Antitumor activity and mechanism of action of ultra-low dose endothelial-monocyte activating polypeptide-II combined with a tumor targeting derivative of TNF

L. Crippa[1], A. Gasparri[1], A. Sacchi[1], E. Ferrero[1], F. Curnis[1], A. Corti[1]
[1]Ospedale San Raffaele, Department of Oncology and IIT Network Research Unit of Molecular Neuroscience, Milano, Italy

Endothelial-monocyte activating polypeptide-II (EMAP-II) is a tumor-derived antiangiogenic cytokine that can sensitize tumors to the damaging activity of high-dose tumor necrosis factor-a (TNF). However, high-dose combination of these cytokines cannot be used for systemic treatment of patients because of prohibitive toxicity. In order to overcome this limitation we investigated the combination of EMAP-II with NGR-TNF, a tumor vasculature-targeted TNF derivative currently tested in Phase II studies. We observed that ultra-low doses of EMAP-II and NGR-TNF can exert synergistic anti-tumor effects and can inhibit the tumor growth in murine RMA-lymphoma and B16-melanoma models, even in the absence of chemotherapy, with no evidence of toxicity. The dose-response curve was bell-shaped, maximal synergism being achieved when 0.1 ng of EMAP-II was given to mice 0.5 hours before 0.1 ng of NGR-TNF. Surprisingly higher doses of EMAP-II in the nanogram/microgram range could progressively inhibit the synergism.Studies on the mechanism of action underlying this synergistic antitumor activity showed that while RMA lymphoma and B16 melanoma tumor cells were resistant in vitro to a wide range of concentrations of EMAP-II and NGR-TNF, low-dose combination of these cytokines induced endothelial cell apoptosis in vivo within 8 hours from administration and, at later time points, caused reduction of vessel density and, in turn, massive apoptosis of tumor cells. Since EMAP-II / NGR-TNF combination could not induce direct cytotoxic effects on cultured HMEC-1 and HUVEC endothelial cells, it is likely that other factors present in the tumor microenvironment are critical for the observed in vivo activity. Vascular targeting of TNF was critical, as the combination of non-targeted TNF with EMAP-II was inactive in these murine models, supporting the hypothesis of vascular damage as the mechanism of action of EMAP-II / NGR-TNF combination. The observation that human as well as murine NGR-TNF can induce marked apoptosis of tumor cells in combination with EMAP-II suggests that TNF-R1 is primarily involved in the pro-apoptotic mechanism, as human TNF binds only TNF-R1.Furthermore, we observed that doses of EMAP-II higher than 1 ng were able to induce the release of soluble TNF-R1, a strong counter-regulatory inhibitor of TNF, accounting for the observed inhibition of the antitumor activity.The combination of NGR-TNF with ultra-low dose EMAP-II could be a new strategy for cancer therapy.

286 Poster

The Vav3 proto-oncogene is a transcriptional target of the dioxin receptor that contributes to fibroblast shape and adhesion

J.M. Carvajal-Gonzalez[1], X.R. Bustelo[2], P.M. Fernandez-Salguero[1]
[1]University of Estremadura, Biochemestry and Molecular Biology, Badajoz, Spain; [2] CSIC-Universidad de Salamanca, Centro de Investigación del Cáncer and Instituto de Biología Molecular y Celular del Cáncer, Salamanca, Spain

Recent studies are uncovering unanticipated roles of the dioxin receptor (AhR) in cell plasticity and migration. Nevertheless, the molecular pathways and the signaling involved remain largely unknown. Here, we report a mechanism that integrates AhR into these cytoskeleton-related functions. Immortalized (FGM) and primary mouse embryonic fibroblasts (MEF) lacking AhR (AhR-/-) had increased cell area and expanded cytoplasms that reverted to wild type spindle-like morphology upon AhR re-expression. The AhR-null phenotype was characterized by an increase in F-actin stress fibers, depolarized focal adhesions, enhanced spreading and attachment and reduced migration. We present evidences that the cytoskeletal alterations observed in AhR-deficient cells are due to diminished expression of Vav3, a GDP/GTP exchange factor for Rho/Rac GTPases and a new transcriptional target of AhR. Dioxin receptor was recruited to the vav3 promoter and maintained its constitutive mRNA expression in a ligand-independent manner. Consistently with these observations, AhR-/- fibroblasts had reduced Rac1 activity and increased activation of the RhoA/Rock pathway. Pharmacological inhibition of Rac1 shifted AhR+/+ fibroblasts to the null phenotype while a Rock inhibitor revert AhR-/- cells to the wild type morphology. Importantly, knockdown of vav3 transcripts by small interfering RNAs in fibroblasts induced cytoskeletal defects and changes in adhesion and spreading that closely mimicked those observed in AhR-null cells. By modulating cell phenotype through this Vav3-dependent pathway, AhR could regulate the shape, adhesion and migration of normal cells and, perhaps, contribute to the abnormal function of these pathways under pathological conditions.

287 Poster

Combined inhibition of vascular endothelial growth factor and overexpression of Angiopoietin-2 enforces glioma regression

D. Eddi[1], S. Valable[1], J.S. Guillamo[1], J. Toutain[1], S. Roussel[1], M. Bernaudin[1], E. Petit[1]
[1]Centre CYCERON, CERVOxy team "Hypoxia and cerebrovascular pathophysiology" UMR 6232 CI-NAPS, Caen, France

Background: Angiogenesis inhibition appears to be promising therapies for glioblastoma, a highly vascularized brain tumor. Sunitinib (SU) is an oral