61 Poster

Heterozygous deficiency of the oxygen sensor PHD2 prevents metastasis by inducing vessel normalization

D. Dettori[1], M. Mazzone[1], R. Leite-de-Oliveira[1], J. Aragones[1], B. Jonckx[1], A. Luttun[1], S. Vinckier[1], B. Jordan[2], B. Gallez[2], P. Carmeliet[1]
[1]Flanders Interuniversity Institute for Biotechnology (VIB) / KULeuven, Transgene Tecnology and Gene Therapy, Leuven, Belgium; [2] Department of Nuclear Medicine, University Hospital, K.U. Leuven, Leuven, Belgium

OBJECTIVES: To study the function of the PHD oxygen sensors in the tumor stroma on tumor growth, angiogenesis and metastasis through in vivo mouse models.MATERIALS: B16 F10 melanoma tumor cells, Panc02 pancreatic tumor cells, LLC Lewis lung carcinoma cells were grown in different media and injected subcutaneously or orthotopically in PHD1-/-, PHD2+/-, PHD3-/- and wild type mice, congenic on a C57Bl6/J background. Mice were generated and bred in our institution. RESULTS: Mice lacking one allele encoding the HIF prolyl hydroxylase PHD2 (PHD2+/-) but not PHD1 and PHD3 deficient mice were resistant to develop metastasis when challenged with a tumor despite a significant increase of the tumor growth. This effect was mediated by the upregulation of the soluble form of VEGFR-1, sFlt1, in endothelial cells that occurred via stabilization of HIF-2, but not HIF-1. sFlt1 acted as an endogenous trap for VEGF-A. This prevented tumor vessel activation and leakage, therefore improving the perfusion of the tumor and reducing the extent of tumor hypoxia. Although favorable for the growth of the primary tumor, this condition dramatically inhibited cancer cell spreading, intravasation and metastatization. CONCLUSIONS: These findings delineate a new role for PHD2 in tumor vessel normalization, and offer a novel mechanistic insight of the anti-angiogenic therapy as a strategy to prevent tumor metastasis.

62 Poster

Tumor stem cells in gliomas - clinical impact of the stem cell marker CD133 and therapeutical strategies

C. Herold-Mende[1], B. Campos[1], F. Wan[1], F. Zeppernick[1], R. Ahmadi[1], W. Roth[2], P. Lichter[3], A. Unterberg[1], B. Radlwimmer[3]
[1]University Heidelberg, Neurosurgery, Heidelberg, Germany; [2] DKFZ, Neurooncology, Heidelberg, Germany; [3] DKFZ, Molecular Genetics, Heidelberg, Germany

A considerable amount of evidence has been gathered supporting the existence of tumor stem cells in a variety of cancers. Glioma-derived tumor stem cells (GTSC) can be enriched by the stem cell surface antigen CD133. Conversely, a controlled, drug-induced depletion of the CD133-positive GTSC pool could have profound therapeutic implications. Retinoids like all-trans retinoic acid (ATRA) have been shown to induce differentiation of GTSCs in vitro. However, it remains unknown whether tumor growth-relevant features of these cells are affected.

We analyzed expression of CD133 in 95 gliomas of various grade and histology by immunohistochemistry. Staining data were correlated with patient outcome. Furthermore, several GTSC lines with high CD133 content were established to investigate ATRA-induced differentiation and potential effects on tumor growth-relevant properties. Proliferation was monitored by BrDU-incorporation assay and CD133 content by FACS-analysis. Impact of differentiation on angiogenic capacity of GTSCs was measured by quantification of angiogenic cytokines and assessed in a HUVEC-based tube formation assay. Potential effects on GTSC invasiveness were studied in a 3D-collagen invasion model. Finally, we studied whether in vitro effects could be confirmed in vivo using a NOD/SCID-mouse xenograft model.

By multivariate survival analysis, both the proportion of CD133-positive cells and their topological organization in clusters were significant ($P < 0.001$) prognostic factors for adverse progression-free (PFS) and overall survival (OS). Also, proportion of CD133-positive cells was an independent risk factor for tumor regrowth and time to malignant progression in WHO II and III tumors. Supporting these clinical data, we present functional evidence that GTSCs exposed to ATRA lower the expression of CD133 in favor of incremented expression of lineage markers. This is accompanied by a significantly reduced VEGF and bFGF secretion, as well as a significantly lowered angiogenic activity following differentiation. Additionally, we show that differentiation elicits strong anti-invasive effects reducing invasion of GTSCs accompanied by a downregulation of invasion-related MMP2 protein. Finally, we report that xenografted tumors of differentiated GTSCs are significantly smaller and less invasive than undifferentiated GTSC tumor xenografts. Correspondingly, animals bearing differentiated cells show both significantly better PFS and OS than mice with GTSC xenografts.

These findings constitute the first conclusive evidence that CD133 expression correlates with patient survival in gliomas, lending support to the current cancer stem cell hypothesis. Additionally, we present functional evidence that differentiation treatment targets the tumor-driving compartment in glioblastoma and constitutes a potential therapeutic approach in the eradication of GTSCs.

63 Poster

The HIF-induced carbonic anhydrase IX and XII regulate intracellular pH promoting tumor survival in a hypoxic and acidic microenvironment

J. Chiche[1], J. Laferrière[1], E. Trottier[1], M.C. Brahimi-Horn[1], J. Pouyssegur[1]
[1]Centre Antoine Lacassagne (CLCC), Institut de Biologie du Développement et Cancer, Nice, France

Acidosis of the tumor microenvironment is typical of a malignant phenotype. Under hypoxic conditions increased lactic acid secretion together with carbonic acid production contribute to a high acid load in the tumor. All cells express multiple isoforms of carbonic anhydrase (CA), zinc-dependent enzymes capable of catalyzing the reversible hydration of carbon dioxide into bicarbonate and protons. The expression of membrane-bound CAs such as CAIX and CAXII is tightly controlled by oxygen levels via the Hypoxia-Inducible Factor (HIF) in many cancers. In particular tumor expression of HIF-1 and CAIX correlates with poor patient survival. What is the contribution of CAIX and CAXII to the maintenance of the intracellular pH (pHi) in an acidic environment? What advantage do tumor cells derive from their overexpression?

To answer these questions: i) we forced the expression of human CAIX or CAXII in fibroblasts that do not express these isoforms and ii) we silenced CAIX expression combined with or without CAXII silencing in two human tumor cell lines (colon adenocarcinoma LS174, melanoma A375). We demonstrate that cells expressing CAIX strongly acidify the extracellular milieu in response to a 'CO2-load' under both hypoxic and normoxic conditions. In hypoxic tumor cells that express both CA isoforms, double silencing is necessary to abolish extracellular acidification in response to a 'CO2-load'. Interestingly, in spite of their capacity to acidify the extracellular milieu, CAIX- or CAXII-expressing cells survive better than control cells. We showed that at low pHe (6 to 7), these cells are able to maintain a resting pHi of 0.2 to 0.3 pH units more alkaline than control cells. Consequently tumor cells expressing high levels of CAIX would survive in an acidic microenvironment much better than normal cells. Preliminary in vivo experiments indicate that CAIX silencing alone leads to a 38% to 45% reduction in the tumor volume. Co-invalidation of both isoforms is in progress. Thus, CAIX and CAXII are major pHi-regulating systems in a tumor hypoxic microenvironment and as pointed out by others, they represent potential targets for anticancer drug development.

64 Poster

Hypoxia-induced autophagy is mediated through the HIF-induction of BNIP3 and BNIP3L via their BH3-domains

N. Mazure[1], G. Bellot[1], R. Garcia-Medina[1], D. Roux[1], J. Pouysségur[1]
[1]Centre Antoine Lacassagne (CLCC), CNRS - UMR 6543, Nice, France

While HIF is a major actor in the cell survival response to hypoxia, HIF is also associated with cell death. Several studies have pointed the implication of HIF-induced putative BH3 only pro-apoptotic genes bnip3 and bnip3L in hypoxia-mediated cell death. We, like others, do not support this assertion. Here we demonstra te that the rapid induction of BNIP3 and BNIP3L in a hypoxic microenvironment contributes to survival rather than cell death by inducing autophagy. First, whereas siRNA mediated ablation of either BNIP3 or BNIP3L had little effect, combined silencing of the two HIF targets suppressed hypoxia-mediated autophagy. Second, ectopic expression of both BNIP3 and BNIP3L in normoxia activates autophagy. Third, 20-mer BH3-peptides of BNIP3 or BNIP3L, modified with a TAT-like membrane transducing sequence were found to be sufficient to activate the autophagy process in normoxia. We propose a model in which the atypical BH3-domains of hypoxia-induced BNIP3/BNIP3L have been 'designed' for inducing autophagy. They disrupt the Beclin1-Bcl2 complex without inducing cell death.

The identification of BNIP3 and BNIP3L as central mediators of autophagy definitively provides new aspects on their functions as pro-survival proteins in opposition to pro-cell death proteins. These results give us good reasons to think that manipulation of HIF-induced autophagy via BNIP3 and BNIP3L may be a good therapeutic option to investigate in cancer treatment.

65 Poster

HIF-1 links bacterial infection, inflammation and cancer

G. Cane[1], A. Ginouvès[2], E. Berra[2], J. Pouysségur[2], P. Hofman[3], V. Vouret-Craviari[3]
[1]Faculte de Medecine Nice Sophia Antipolis, ERI21, Nice, France; [2] Faculte de Sciences Nice Sophia Antipolis, UMR6543, Nice, France; [3] Faculte de Médecine Nice Sophia Antipolis, ERI21, Nice, France

Background: Infection of human intestinal cells with Afa/Dr DAEC C1845 pro inflammatory bacteria induces expression of the VEGF gene, encoding Vascular Endothelial Growth Factor.

Materials and Methods: Expression of HIF-1alpha was assayed by Werstern Blotting and qPCR. Transfection of siRNA against HIF-1alpha was done to demonstrate its transcriptional activity. A pull down assay using as bait the oxygen binding domain of HIF-1 was used to assay PHD activity.

Results: Here, we demonstrate that exposure of T84 colon cancer cells to C1845 bacteria induces the expression of HIF-1, a key transcription factor involved in VEGF expression. In contrast to hypoxia which inhibits the activity of prolyl hydroxylases (PHD) and as a consequence stabilizes HIF-1alpha protein, C1845 bacteria do not inhibit PHD activity but rather induce translational mechanisms. C1845 stimulation of HIF-1alpha required the binding of F1845 adhesin to the apical DAF/CD55 receptor. HIF-1alpha expression was inhibited by treating the cells with inhibitors of Src like tyrosine kinase, MAP kinase and phosphatidylinsositol 3-kinase signaling pathways. These inhibitors also blocked the C1845-induced phosphorylation of the translational regulatory protein p70 S6 kinase thus providing a mechanism for the modulation of HIF-1alpha protein synthesis. In addition to VEGF, C1845 bacteria induce the expression of BNIP3, a major regulator of autophagy. Autophagy is a process by which cytoplasmic organelles can be catabolized to provide macromolecules for energy generation under conditions of nutrient starvation.

Conclusion: Thus we propose that C1845-induced HIF1alpha expression could promote the survival of human colon cancer cell.

66 Poster

A crosstalk between HIF-1a and LOX in the tumor microenvironment

F. Pez[1], F. Dayan[2], J. Durivault[2], B. Kaniewski[1], C. Reynaud[1], J. Pouysségur[2], P. Sommer[1]
[1]Institut de Biologie et Chimie des Protéines IBCP, Cnrs, Lyon, France; [2] Institute of Developmental Biology and Cancer, Cnrs, Nice, France

The Lysyl oxidase gene family (LOs) comprises five members acting as extracellular modulating enzymes. Lysyl oxidase (LOX), the first member of the family, catalyzes the cross-linking of collagen and elastin and its expression correlates with metastatic potential in tumor cell lines. Importantly, recent data revealed an overexpression of LOX under hypoxic conditions. This up-regulation is under the control of the Hypoxia-Inducible Factor-1a (HIF-1a), a key transcription factor involved in cellular adaptation to changes in O2 level. In addition to LOX, our results suggest that other LOs isoforms are regulated by hypoxia in several tumorigenic cell lines, confirming the tight control of LOs by the cancer micro-environment. Reciprocally, we pointed out that LOX can also act on the HIF-1a pathway. We showed this new link using human colorectal carcinoma cell lines in which the expression of LOX is modulated under both normoxic and hypoxic conditions. Indeed, LOX is able to regulate the expression of HIF-1α protein, as well as the downstream effector Carbonic Anhydrase IX. Taken together these results underline an inter-relation and a positive feedback loop between two main actors of tumoral progression: HIF-1a and LOX.

67 Poster

Role of lymph vessels in progression of breast cancer; morphological characteristics and prognostic implication

R.A.A. Mohammed[1], S. Elsheikh[2], M.S. Gill[3], A.R. Green[2], E.C. Paish[4], I.O. Ellis[4], S.G. Martin[5]
[1]University of Nottingham/City Hospital Campus, Clinical Oncology/Pathology, Nottingham, United Kingdom; [2] University of Nottingham/Queen Medical Centre, Pathology, Nottingham, United Kingdom; [3] City Hospital NHS Trust, Pathology, Nottingham, United Kingdom; [4] University of Nottingham/City Hospital Campus, Pathology, Nottingham, United Kingdom; [5] University of Nottingham/City Hospital Campus, Clinical Oncology, Nottingham, United Kingdom

Background: In spite of the growing evidence about the important role of lymphatics in progression of breast cancer, this issue is still a matter of controversy. Aims of the following study were (a) to investigate lymphatic characteristics (lymph vessel density (LVD) and lymphovascular invasion (LVI)) in breast cancer and their role as prognostic factors (b) to study the role of vascular endothelial growth factors (VEGF)-A, VEGF-C and VEGF-D in regulation of lymphangiogenesis (C) to distinguish between LVI and blood vascular invasion (BVI) to find which type of vessels play the major role in metastasis.

Materials and methods: Paraffin embedded sections of 177 invasive breast cancer, with 10 years follow up, were stained immunohistochemically with the lymphatic markers, podoplanin and D-40 to assess LVD and LVI, with CD34 and CD31 to identify BVI and with VEGF-A, VEGF-C and VEGF-D. LVD, LVI and expression of growth factors were correlated together and with survival. Ethical approval was obtained for the study from Nottingham Local Research Ethics Committee.

Results: in breast cancer the majority of lymphatics are located in the peripheral and the peritumoural areas. Tumours with higher LVD are significantly associated with the presence of LN metastasis (P<0.001) and shorter overall survival (OS) (P=0.04). High expression of VEGF-A and -C but not of VEGF-D were associated with high LVD (P= 0.047, <0.001 and 0.187 respectively) and with poorer survival. Vascular invasion was detected in 56/177 specimens (31.6%); 54 (96.4%) were LVI and 2 (3.5%) were BVI. The presence of LVI was significantly associated with the presence of LN metastasis, development of distant metastasis, regional recurrence and worse disease free interval (DFI) and OS. In multivariate analysis LVI but not LVD was an independent poor prognostic factor.

Conclusion: lymphatics in breast cancer play an essential role in disease progression by being the major routs of dissemination. VEGF-A and VEGF-C are important factors for lymphangiogenesis.

68 Poster

Non small cell lung cancer xenografts as preclinical models for epidermal growth factor receptor (EGFR) - targeted therapies

D. Behrens[1], J. Rolff[1], J. Merk[2], S. Lee[3], R. Soong[3], M. Becker[1], I. Fichtner[1]
[1]Max-Delbrueck-Center for Molecular Medicine, Experimental Pharmacology, Berlin, Germany; [2] Evangelische Lungenklinik, Oncology, Berlin, Germany; [3] National University of Singapore, 10 Medical Drive, Berlin, Singapore

Background: The EGFR plays a crucial role in human cancer. It is involved in tumor development and progression, cell proliferation and regulation of apoptotic cell death. In lung cancer the EGFR is frequently overexpressed in 50-80% of the patients. With the tyrosine kinase inhibitors (TKI) Gefitinib and Erlotinib as well as with the monoclonal antibody Cetuximab targeted drugs are available for the treatment of patients with lung cancer. The evaluation of clinical trials using Erlotinib and Gefitinib revealed that only a small group (adenocarcinomas, women, never-smokers and people with asian origin) did benefit from the treatment with TKIs. In addition, the role of mutations in the exon 18- 21 of the EGFR gene was widely investigated and debated.

Method: Up to now, in our group 101 tumors had been transplanted from which 25 transplantable models were generated.

Results: It could be demonstrated that the murine passages coincide with the original tumor regarding histology, the expression of the surface proteins E-Cadherin, EpCAM, the cell proliferation marker Ki-67 and in gene profiling. The analysis of the EGFR gene revealed no mutations relating to a better response to TKIs. With the exception of five models all express a wild type EGFR. Five K-ras mutations were found in the xenografts and 11 different mutations could be located in the p53 gene. Furthermore, the sensitivity of the xenografts was tested against five clinically used cytotoxic agents (Etoposid, Carboplatin, Gemcitabine, Paclitaxel and Navelbine) and two EGFR inhibitors (Erlotinib and Cetuximab). It could be shown that there exist strong differences in responses among the xenografts.

Conclusion: In summary, we have available a panel of well characterized NSCLC xenografts correlating with the clinical situation and being able to identify biomarkers and their regulation after therapeutic interventions both at genetic and at protein level.

69 Poster

Microarray analysis and functional studies in a novel human colon cancer model of EMT: TAF12 regulates E-cadherin and Fra-1 regulates Vimentin expression

A. Pintzas[1], T. Joyce[1], A. Voulgari[1], M. Kalogeropoulou[1], C. Andreolas[1], I. Davidson[2], L. Tora[2], E. Medico[3]
[1]National Hellenic Research Foundation, Institute of Biological Research and Biotechnology, Athens, Greece; [2] Institut de Génétique et de Biologie Moléculaire et Cellulaire, Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; [3] Institute for Cancer Research and Treatment, University of Torino, Torino, Italy

Background: The process of epithelial mesenchymal transition, is a fundamental process of embryogenesis and cancer invasion/metastasis. TFIID is composed of the TATA box-binding protein (TBP) and its associated factors (TAFs). Interestingly, the TFIID activity can be regulated by cellular signals to specifically alter transcription of particular subsets of genes.

Materials and methods. In order to examine the distinctive functions in cancer development in the colon, we introduced constitutively active mutant Ras genes into an intermediate stage colon adenoma cell line (Caco-2).

Results. We found that Ha-RasV12 was very efficient in transforming these cells, which developed a mesenchymal morphology. We conducted microarray analysis in an attempt to reveal the genes whose aberrant expression is a direct result of overexpression of either Ki-RasV12 or Ha-RasV12 (1) and then arrays of more than 25,000 genes (2). We present that vimentin, a key molecule of epithelial mesenchymal transition, was