427 The Major Vault Protein (MVP) Mediates Starvation Resistance of Human Glioblastoma Cells Via Deregulation of the PI3kinase Pathway

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Background: Vaults are highly conserved ribonucleoprotein particles ubiquitously expressed in eukaryotic organisms. They predominantly consist of the 110 kDa major vault protein (MVP) and have been implicated in the regulation of multiple cellular processes including transport mechanisms, chemoresistance, and intracellular signalling pathways. While in normal brain the expression is low, MVP levels are consistently upregulated in glioblastoma multiforme (GBM). Aim of this study was to investigate whether MVP/vaults have an impact on GBM cell growth and survival, including chemotherapy responsiveness, and to clarify underlying molecular mechanisms.
Material and Methods: The MVP protein was stably overexpressed in MVP-low H7 glioma cells. Ectopic and endogenous MVP expression was repressed by MVP mRNA-specific shRNA. Protein expressions were detected by immunofluorescence and Western blot. Consequences of MVP modulation on cell proliferation, survival, chemotherapy response and serum starvation with or without growth factor stimulation were analysed. Additionally, impact of MVP on subcutaneous and orthotopic tumour formation in SCID mice was tested.
Results and Discussion: Ectopic MVP expression in H7 glioma cells did not substantially alter sensitivity against diverse chemotherapeutic drugs. However, responsiveness to growth factor stimulation (EGF, serum) was increased paralleled by a significant upregulation of MAPK- and PI3K-pathway indicated by phosphorylation of ERK, AKT and S6. Moreover, MVP-transgenic cells were impressively resistant to apoptotic cell death induced by serum-starvation, an effect reversible by shRNA-mediated MVP-repression. PI3K downstream signalling, namely AKT and S6 phosphorylation, was hyperactivated in MVP-positive as compared to control transfected cells. Accordingly, inhibition of mTOR via temsirolimus or PI3K via LY-294002 induced both complete blockade of S6 and 4EBP phosphorylation and restoration of apoptosis induction by serum starvation of MVP-positive cells. Subcutaneous tumor growth in SCID mice was significantly enhanced, PI3K signalling distinctly increased and the apoptotic cell fraction reduced in orthotopic xenografts from MVP-overexpressing subclones as compared to vector controls.
Conclusion: Our data proof a significant contribution of vaults/MVP to the malignant phenotype of human GBM cells by supporting enhanced survival under nutrient starvation based on the deregulation of oncogenic signalling pathways.

429 Tumor Resistance to Anti-angiogenic Therapies in Renal Cell Carcinoma Tumorgraft Mouse Models

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Background: Different types of tumors are currently treated with VEGF-targeting therapy as single therapy or in combination with chemotherapy, such as renal cell carcinoma (RCC). Nevertheless, clinical benefit of VEGF signalling inhibitors is short-lived and these therapies fail indeed to produce durable effects and to significantly modify the patient's long-term survival due to tumor adaptation and subsequent resistance to therapy.
Material and Methods: To investigate the mechanisms of resistance in a clinically relevant tumor, we have developed several Tumorgraft mouse models based on the orthotopic implantation of renal tumors derived from primary biopsies of human RCC tumors. We have evaluated the effects of VEGF signalling inhibitors of the murine VEGFR2 (DC101) or the human VEGF ligand (Bevacizumab) after short and long term treatment, on the tumor microenvironment by the analysis of CD31 expression, hypoxia and necrosis.
Results: Our preliminary results showed that the inhibition of VEGF-pathway in a short term therapy affects some biological features of the tumors: decrease of vascular density, increase of hypoxia and necrotic areas of tumor microenvironment thus inhibiting the tumor growth of treated mice compare to the control. In the long-term therapy however we observed a tumor rebound due to the adaptation to treatment associated or not with an increase of vessel density. Ongoing experiments of molecular and immunohistochemical characterization will define the molecular mechanism of acquired resistance to the therapy.
Conclusions: Short-term therapy with DC101 and Bevacizumab exerts an anti-angiogenic effect on RCC tumors that leads to a delay in tumor growth. Long-term therapy fails to control the tumor growth with an eventual rebound of tumor growth due to resistance to anti-angiogenic treatment. Results from new Tumorgraft RCC models based on primary human tumors could have relevant clinical implications in the understanding of the mechanisms involved in the acquisition of resistance to VEGF-pathway inhibition therapy in human patients.

431 Opposite Roles of Embryonic EMT-inducers in B-Raf-driven Melanocyte Transformation

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Originally depicted as poor prognosis factors in light of their prometastatic potential, embryonic EMT inducers additionally behave as determinant drivers of the neoplastic transformation. Although numerous signals are likely to contribute to their aberrant reactivation during tumor progression, a growing body of evidence supports the hypothesis that their induction might be dictated by the initial mitogenic insult. We herein demonstrate that B-Raf activation, as recurrent genetic event in melanomas, induces a drastic upregulation of Zeb1 and Twist1 at the expanse of Zeb2 and Snai2 in murine and human melanocytes. This reprogramming is determinant for B-Raf in promoting cell transformation, likely through the deregulation of cell differentiation/proliferation balance. Immunohistochemical expression analyses on a cohort of human nevi and melanomas supported the observed shift suggesting that EMT inducers either behave as oncogenes or tumor suppressor genes, according to the cellular context.

433 Serum Polyamines in Patients With Non-Hodgkin's Lymphoma

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Background: The polyamines (PAs) putrescine (Put), histamine (His), spermidine (Spd) and spermine (Spm) are a group of naturally occurring compounds that are essentially involved in cell growth and differentiation, with especially elevated levels in fast growing tissues like cancer. They are studied as potential tumour markers. The non-Hodgkin lymphomas (NHLs) are a diverse group of blood cancers that include any kind of lymphoma except Hodgkin's lymphomas, and vary significantly in their severity.
As malignant diseases are in expansion, the aim of our work was to investigate polyamine levels, as dansylated derivatives, inserum of NHL patients, using LC/DAD technique, so we could apply it in clinical practice.
Material and Methods: This study involved sera of 12 patients with NHL (one with T type lymphoma, one with MALT typelymphoma, two with follicular type lymphoma, and eight with diffusion large B cellstype lymphoma), and of 13 healthy volunteers. We precipitated serum proteins using 0.4 M $HClO_4$. At pH 8.0 we performed derivatization with dansyl-chloride. 50 μL of prepared serum samples were injected into LC/DAD, in conditions of gradient elution, on C18 column. Commercially available Put, His, Spd, and Spm were dissolved in different concentrations in ultra pure water; treated in the same way as serum samples and injected into LC for obtaining calibration curves by plotting the PAs peak area values against the respective concentrations of standards. The qualitative analysis was done using the method of retention time. Quantitative analysis was done using the method of external calibration.
Results: Retention times were 9.1 min for Put, 10.1 min for His, 13.2 min for Spd, and 15.4 min for Spm, respectively. Obtained data showed good linearity of calibration curves for Put, His, Spd, and Spm ($R^2 = 1.0$; $R^2 = 1.0$; $R^2 = 0.99997$; $R^2 = 0.99985$, respectively). It is noticed that concentrations of some special polyamines are very changed in some patients with NHL, compared with healthy subjects.
Conclusions: Concentration of polyamines in patients with NHL should be investigated depending on the type of NHL.

434 Embryonic Transcription Factors, MiRNAs and Mitogenic Stresses Network in Breast Tumorigenesis – Deciphering the Interactome

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Background: Reactivation of embryonic programs through Epithelial-to-Mesenchymal Transition (EMT) is associated with tumour initiation and progression endowing tumour cells with self-renewal potential and invasive properties. EMT is associated with a profound genetic reprogramming