The Major Vault Protein (MVP) Mediates Starvation Resistance of Human Glioblastoma Cells Via Deregulation of the PI3K/Akt Pathway

D. Lötsc1, S. Spiegler-Kreinecker2, C. Pirker1, J. Hlavaty3, H. Petnez3, M. Grusch3, W. Berger1. 1Medical University of Vienna, Internal Medicine I / Institute of Cancer Research, Vienna, Austria, 2Wagner-Jauregg Hospital, Department of Neurosurgery, Linz, Austria, 3University of Veterinary Medicine, Institute of Virology / Department of Pathobiology, Vienna, Austria.

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 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, MVP overexpression led to a significant increase in proliferation and survival rates compared to parent cells. 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 resumption of apoptosis induction by serum-starvation of MVP-positive cells. Subcutaneous tumor growth in SCID mice was significantly increased, 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 proves 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.

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

G. Jimenez1, M. Martinez1, L. Moserle1, A. Vidail1, O. Casanova1. 1Instituto Catalán Oncología, LRT1, Barcelona (Barcelona), Spain, 2Bellvitge University Hospital, Anatomopathology Department, Barcelona (Barcelona), Spain.

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 signaling 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 signaling 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 compared 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.

Conclusion: 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.