and EGFR expression was observed in GICs but not in differentiated cells. Withdraw of EGF from growth medium of GICs decreased sox2 and nestin mRNA. Knockdown of EVI1 in GICs decreased EGFR and nestin mRNA.

**CONCLUSION:** EVI1 maintains undifferentiated status of GICs through transcriptional regulation of EGFR gene expression.

**P001.07 IDENTIFICATION OF NOVEL MOLECULAR TARGETS FOR TMZ-BASED THERAPIES AGAINST GliOBLASTOMA: A COMPREHENSIVE SHRNA-BASED SCREEN OF DNA DAMAGE RESPONSE FACTORS**

H. Erasmus1, S. Fritsche1, S. Haas1, C. Herold-Mende1, B. Klink1, J. F. Masson1, P. V. Nazarov2, S. P. Nicolau3, E. Van Dyck1

1NorLux Neuro-Oncology Laboratory, Luxembourg Institute of Health, Luxembourg, Luxembourg; 2Life Sciences Research Unit, University of Luxembourg, Luxembourg, Luxembourg; 3Deutsches Krebsforschungszentrum, Heidelberg University Hospital, Heidelberg, Germany.

Despite surgical resection and genotoxic treatment with ionizing radiation and the DNA alkylating agent temozolomide (TMZ), glioblastoma (GBM) remains one of the most lethal cancers, due in part to the action of DNA repair factors that drive resistance and lead to tumor relapse. Improved outcomes in these patients do not prolong overall survival: gliomas acquire resistance to TMZ and exhibit inherent redundancy and complexity of the many DNA repair pathways activated as part of the DNA damage response (DDR) to DNA damage. Understanding how DNA repair mechanisms operate to remove lesions induced by TMZ in GBM cells and identifying novel DDR targets remain of vital importance in order to improve GBM treatment.

Our experimental strategy to reach these goals consisted in large-scale, loss-of-function shRNA screens for DDR genes that are required for the survival of GBM cells to TMZ. Pooled shRNAs targeting more than 500 DDR genes were introduced into selected GBM-derived, cancer stem-like cells, as well as into control non-cancer cells, followed by identification of the genes that appear essential to the survival of these cells, but dispensable to the survival of GSC. Two major screens were carried out to reflect the clinical benefit conferred by epigenetic inactivation of the MGMT gene encoding a crucial factor for the repair of TMZ-induced lesions, on the response of GBM patients to TMZ - one with MGMT-positive cells and one where MGMT was depleted through pharmacological inhibition. The first goal has been reached and potential DDR targets that could sensitize cancer cells to TMZ have been identified.

We will now validate a very promising gene, in vitro as well as in vivo in pre-clinical models of GBM, and elucidate the molecular mechanisms by which this gene operates to confer cellular resistance to TMZ.

**P001.08 TARGETING DNA REPAIR MECHANISMS IN GliOBlastoma: FROM BASIC MECHANISMS TO PRE-CLINICAL ASPECTS AND PERSONALIZED THERAPY**

E. Van Dyck1, H. Erasmus1, M. Gobin1, P. Nazarov2, S. Fritsche1, L. Vallar3, M. Timmer4, R. Goldbrunner5, S. Nicolau1

1Neuro-Oncology Laboratory, Luxembourg Institute of Health, Luxembourg, Luxembourg; 2Genomics Research Group, Luxembourg Institute of Health, Luxembourg, Luxembourg; 3Neuro-Oncology Department, University of Cologne, Köln, Germany.

INTRODUCTION: Despite surgical resection and genotoxic treatment with ionizing radiation (IR) and the DNA alkylating agent temozolomide (TMZ), glioblastoma remains one of the most lethal cancers, due in great part to the action of complex DNA repair mechanisms that drive resistance and tumour relapse. One such mechanism involves the DNA-repair protein O6-methylguanine-DNA methyltransferase (MGMT) which mediates the direct removal of O6-methylguanine, the most highly cytotoxic lesion induced by TMZ. Importantly, silencing of MGMT through promoter methylation, which is observed in about 40% of GBM patients, confers a small but significant survival benefit in patients treated with TMZ and IR compared to IR only. In addition to MGMT, several DNA repair pathways have been involved in the repair of TMZ-induced lesions. Understanding the molecular details of these mechanisms and identifying potential pharmacological targets have emerged as vital tasks to improve treatment. At the same time, deciphering the genetic and epigenetic alterations that shape the “DNA repair makeup” of GBM cells should help tailor therapy to individual patients.

**APPROACH AND RESULTS:** We have undertaken complementary approaches to understand the molecular basis behind chemoresistance, tumour progression and relapse in GBM patients. Firstly, we have meas-
ured the mRNA expression levels of a selection of DNA repair and cell cycle factors in paired, primary and recurrent GBM biopsies from patients treated or not with TMZ. Classification of the deregulated genes led to the identification of a gene signature that segregated 3 groups of biopsies. Inspection of these groups suggests that one route to tumour progression is associated with profound alterations in cell cycle genes as well as genes encoding crucial DNA repair factors. We have further explored our differential gene expression analysis to reveal the expression of specific pathways in response to glioblastomaogenes and genotoxic treatment, and propose novel therapeutic strategies based on the inhibition of DNA repair factors. Lastly, we have carried out a large-scale shRNA screen of DNA repair and proliferation factors to identify those gene silencings that sensitize GBM cells to TMZ, as well as synthetic lethal interactions with MGMT. We are currently validating the most promising candidates in vitro as well as in vivo, using orthotopic xenograft models of GBM.

RESULTS: Differential gene expression of several activators (i.e. CASP1, TLRs, BIRC3, IL1B), inhibitors (NFKBIE, TNFAIP3) and downstream targets (i.e. IFNG, CD83) of the NF-κB pathway was observed after VPA treatment in vitro. VPA inhibited NF-κB transcriptional activity after 48 hours. In addition, VPA increased expression levels of acetylated histones H3 and H4 in vitro, but had no effect on direct acetylation of p65 or TCF transcriptional activity. In tumor samples obtained from glioblastoma patients however, we did not observe any immunohistochemical evidence of an effect of VPA treatment on NF-κB activation or histone acetylation.

CONCLUSION: At a clinically relevant concentration, VPA targets the NF-κB signaling pathway at multiple levels, and inhibits NF-κB transcriptional activity in vitro. This effect is most likely mediated by HDAC inhibition. However, in tumor samples from glioblastoma patients, these effects are not observed. Current clinical dosages of VPA might not be sufficient to mediate significant biological effects in the tumor tissue in patients, which might explain the lack of effect of VPA on clinical outcome.

P01.09 EPILEPSY ASSOCIATES WITH DECREASED HIF1-α/STAT5B AND SRF SIGNALING IN GliOBLASTOMA
S. Berendsen1,2, W. G. M. Spliet1, M. Geurts3, W. Van Hecke1, T. Seute1, T. J. Snijders1, E. H. Bell1, A. Chakravart1, P. A. Robe1,2,3,4,5
1University Medical Center Utrecht, Utrecht, Netherlands, 2Ohio State University, Columbus, OH, United States, 3University Hospital of Liege, Liege, Belgium.

BACKGROUND: We have found that epilepsy at presentation is a favorable prognostic factor in glioblastoma. In this study, we analyze the biological mechanisms that associate with epilepsy in glioblastoma patients, and which might thus underlie this prognostic effect.

MATERIALS AND METHODS: Hospital records were screened for the presence of seizures at presentation of the disease in a cohort of 647 patients. Protein expression patterns of gene set targets (ATRX, STAT5B, VEGF) and markers of epithelial-mesenchymal transition (NF-κB, C/EBP-β and STAT3) were determined with immunohistochemistry on tissue microarrays containing 339 tumors. Molecular classification, mRNA and miRNA expression-based (gene set) enrichment analyses were performed on fresh frozen tissue obtained from a subset of 76 tumors.

RESULTS: Gene sets involved in hypoxia/HIF1-α, SRF, C/EBP-β and LPS-induced signaling pathways were significantly downregulated in glioblastoma patients who presented with epilepsy. On an immunohistochemical level, epileptogenic tumors were characterized by a significant downregulation of phospho-STAT5B, a target of HIF1-α. No differential expression between patients with or without epilepsy was observed for VEGF or ATRX, which is a target of SRF and has been associated with prognosis of glioma patients. Epilepsy status did not associate with EMT protein expression, molecular classification or miRNA expression.

CONCLUSIONS: Epileptogenic GBMs show a downregulation of HIF1-α/STAT5B and SRF signaling compared to glioblastomas that do not present with epilepsy. These genes are known to play oncogenic roles in glioblastoma and other types of cancer, and SRF, which is deregulated in non-tumoral epileptic brain, might provide insight in the tumor’s epileptogenicity.

P01.10 EFFECTS OF VALPROIC ACID ON NF-kB SIGNALING IN GLIOBLASTOMA
S. Berendsen1, M. Frijlink1, J. Kroonen1,5, W. G. M. Spliet1, W. Van Hecke1, T. Seute1, T. J. Snijders1, P. A. Robe2,3
1University Medical Center Utrecht, Utrecht, Netherlands, 2University Hospital of Liege, Liege, Belgium.

INTRODUCTION: Aberrant activation of NF-kB signaling in glioblastoma has been linked to mesenchymal activation, resistance to chemotherapy and increased invasion. The HDAC inhibitor and anti-epileptic drug valproic acid (VPA) has been suggested to inhibit NF-kB activity in certain cell types. In this study, we analyze the effects of VPA treatment on NF-kB signaling in glioblastoma and investigate the underlying mechanisms of this effect.

MATERIALS AND METHODS: We analyzed the effects of VPA treatment on NF-kB signaling in glioma cell lines and primary cell cultures with qPCR arrays and reporter gene assays. Histone and p65 acetylation levels were analyzed by Western blotting. Involvement of the GSK-3β/b-catenin pathway was assessed with kinase and reporter gene assays. With use of immunohistochemistry and Western blotting we then investigated expression levels of acetylated histones and phospho-p65 in tumor tissues obtained from glioblastoma patients who were either treated with VPA or did not receive anti-epileptic drugs at the time of their surgery.

RESULTS: Valproic acid reduced NF-kB signaling and phosphorylation of the proto-oncogene cJun. However, this effect was not observed when the HDAC inhibitor acts on cells expressing low levels of HDAC6. Protein expression patterns of gene set targets (ATRX, STAT5B, VEGF) and markers of mesenchymal transition (NF-κB, C/EBP-β and STAT3) were determined with immunohistochemistry on tissue microarrays containing 339 tumors. Molecular classification, mRNA and miRNA expression-based (gene set) enrichment analyses were performed on fresh frozen tissue obtained from a subset of 76 tumors.

CONCLUSIONS: Epilepsy at presentation in glioblastoma patients who were either treated with VPA or did not receive anti-epileptic drugs at the time of their surgery. VPA inhibited NF-kB signaling and increased invasion. The HDAC inhibitor and anti-epileptic drug valproic acid after 48 hours. In addition, VPA increased expression levels of acetylated histones H3 and H4 in vitro, but had no effect on direct acetylation of p65 or TCF transcriptional activity. In tumor samples obtained from glioblastoma patients however, we did not observe any immunohistochemical evidence of an effect of VPA treatment on NF-kB activation or histone acetylation.

CONCLUSION: At a clinically relevant concentration, VPA targets the NF-kB signaling pathway at multiple levels, and inhibits NF-kB transcriptional activity in vitro. This effect is most likely mediated by HDAC inhibition. However, in tumor samples from glioblastoma patients, these effects are not observed. Current clinical dosages of VPA might not be sufficient to mediate significant biological effects in the tumor tissue in patients, which might explain the lack of effect of VPA on clinical outcome.

P01.11 THE TRANSCRIPTIONAL CO-ACTIVATOR PGC-1α PROMOTES DEFENCE AGAINST REACTIVE OXYGEN SPECIES (ROS) AND PROLIFERATION IN HUMAN GliOMA CELLS
J. Berends1, M. Sauer1, M. Burger1, M. W. Romelienfus1, J. P. Steinbuch1,2, E. Rüger1
1Dr. Senckenberg Institute of Neurooncology, University Hospital Frankfurt, Frankfurt am Main, Germany, 2German Cancer Consortium (DKTK), Heidelberg, Germany.

Gene expression of many signalling pathways and metabolic programs is regulated at the transcriptional level by DNA binding coactivators. These coactivators act in a tissue-specific manner and are responsive to different physiological stimuli like nutrient supply and temperature. One of these coactivators, Peroxisome proliferator-activated receptor coactivator (PGC)-1α, is a master regulator of mitochondrial biogenesis, controls antioxidative responses and coordinates posttranscriptional events. The activity of PGC-1α is regulated by growth factor receptor signaling and the AMP-activated protein kinase (AMPK) pathway, both of which are crucial for the growth and metabolism of tumors. Further, because variable nutritional environments require tumor cells to adapt their energy metabolism, we asked whether PGC-1α is involved in growth, resistance against nutrient depletion and metabolic flexibility of glioma cells.

We found that PGC-1α is expressed in a subset of established and primary glioma cells. Surprisingly, while suppression of PGC-1α expression by shRNA in U343MG glioma cells reduced glucose consumption and lactate production, it did not reduce oxygen consumption or mitochondrial mass. Compatible to the known PGC-1α functions in reactive oxygen species (ROS) metabolism, glioma cells lacking PGC-1α expression had reduced RNA levels of proteins involved in (ROS) detoxification mechanism, and these cells were more susceptible to death induced by H2O2 led to cell death. Interestingly, the PGC-1α knock-down conferred protection against hypoxia-induced cell death. In an in vivo xenograft experiment, tumors formed by PGC-1α-suppressed glioma cells grew much slower than control tumors.

In summary, PGC-1α normally plays a crucial role in growth and resistance against ROS in these cells. As the oxygen consumption in PGC-1α-sh cells was not significantly impaired, the presented effects of PGC-1α seems not to depend on the well-established functions of PGC-1α on mitochondrial oxidative phosphorylation. Further experiments are underway to analyse in more detail the metabolic and cellular functions of PGC-1α.

P01.12 VEGF-C CONTRIBUTES TO AUTOCRINE VEGF RECEPTOR 2 SIGNALING AND CELLULAR MALIGNANCY IN GLIOBLASTOMA
S. R. Michaels1, M. K. Nedergaard2, H. Broholm1, J. Bartkova1, M. Staberg1, M. Aghi1, S. Lucacova4, I. Perryman1, H. S. Poulsen1, P. Hamerlik1
1Department of Radiation Biology, Section 6321, Rigshospitalet, Copenhagen, Denmark, 2Department of Clinical Physiology, Nuclear Medicine & PET and Cluster for Molecular Imaging, Rigshospitalet and University of Copenhagen, Copenhagen, Denmark, 3Department of Pathology, Rigshospitalet, Copenhagen, Denmark, 4Danish Cancer Society Research Center, Copenhagen, Denmark, 5Department of Neurological Surgery, University of California, San Francisco, CA, United States, 6Aarhus University Hospital, Aarhus, Denmark, 7BRIC, University of Copenhagen, Copenhagen, Denmark.

INTRODUCTION: Despite high expression of the angiogenic stimulator VEGF-A and recently reported autocrine VEGF-A-VEGFR2 signaling