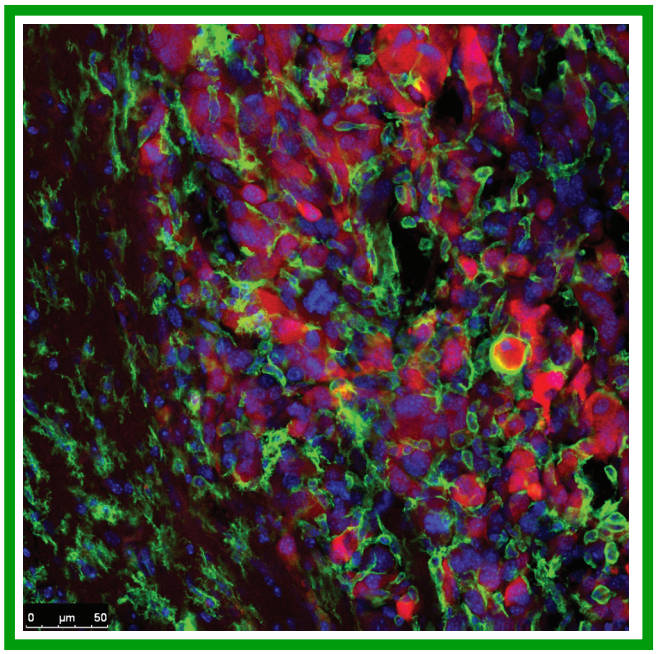
Brain Tumor Meeting 2017



Program and Abstracts
(Plenaries, Orals and Posters)

May 18 - 19, 2017

Campus Berlin-Buch
Max Delbrück Communications Center (MDC.C)
Robert-Rössle-Str. 10
D-13125 Berlin, Germany

This meeting is an activity of

MDC MAX-DELBRÜCK-CENTRUM FÜR MOLEKULARE MEDIZIN BERLIN-BUCH in der HELMHOLTZ-GEMEINSCHAFT e.V.

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

HELIOS Kliniken Jeder Moment ist Medizin

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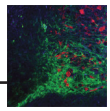
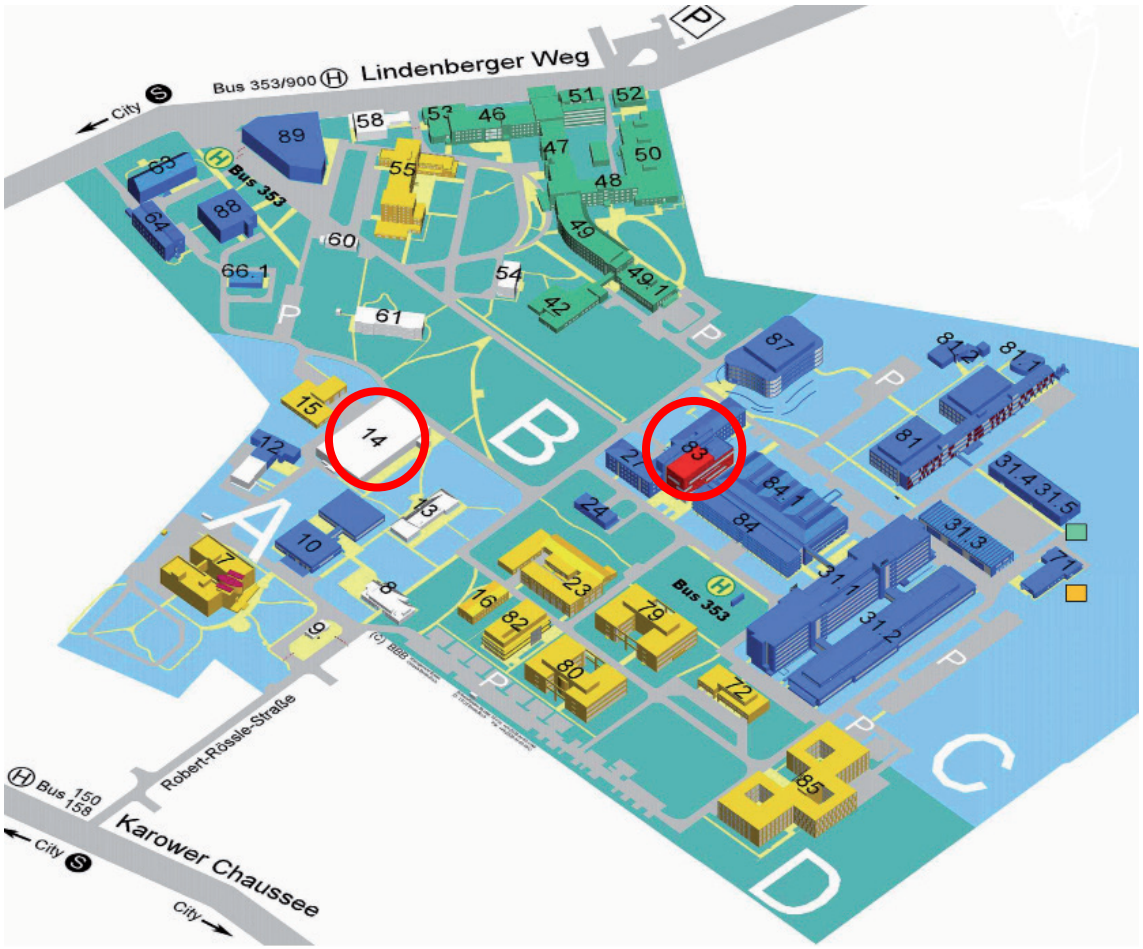
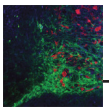


Campus Berlin-Buch



A9 - Pförtner
A8 - Torhaus
A15 - Charles River Deutschland GmbH
A14 - Mensa
A13 - Infocenter, Gläsernes Labor
A10 - Bibliothek
B64 - epo GmbH
B63 - Tierhaus
B61 - Salvadore-Luria-Haus
B55 - OCVH - Oskar-und-Cécile-Vogt-Haus
B54 - Hans-Gummel-Gästehaus
B46 - Robert-Rössle-Klinik
C27 - Walter-Friedrich-Haus
C87 - Timoféeff-Ressovsky-Haus

C31.1- MDH
C31 - MDH - Max-Delbrück-Haus
C84 - Hermann-von-Helmholtz-Haus
C83 - MDC.C
C81 - FMP
C71 - Tier- und Laborgebäude
D85 - Arnold-Graffi-Haus
D82 - Karl-Lohmann-Haus
D80 - Otto-Warburg-Haus
D79 - Erwin-Negelein-Haus
D72 - Haus 72
D23 - Eckert & Ziegler AG
D16 - Bebig GmbH



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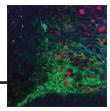
Scientific Committee



Acknowledgement

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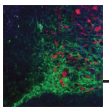


Scientific Program

Thursday, May 18, 2017

14.00 –-14.05 **Welcome Address:** Helmut Kettenmann

14.05 - 15.25 **Session I**
Chair: Jürgen Kiwit

14.05 - 14.45 **Plenary Lecture I**
Eric Charles Holland (Seattle, USA)
Gliomas: Big human data and mouse models

14.45 - 15.05 **Oral Presentation I**
Roberto Fiorelli (Phoenix, USA)
Molecular and cyto-architectonic reshaping of the human svz during glioma invasion

15.05 - 15.25 **Oral Presentation II**
Dinorah Friedmann-Morvinski (Tel Aviv, Israel)
Functional characterization of oncogenic-induced cell pasticity in glioblastoma

15.25 - 16.00 **Poster Session and Coffee Break**

16.00 - 17.20 **Session II**
Chair: Susanne Wolf

16.00 - 16.40 **Plenary Lecture II**
Cameron W. Brennan (New York, USA)
Molecular heterogeneity and instability of diffuse gliomas: models and clinical practice

16.40 - 17.00 **Oral Presentation III**
Cécile Maire (Hamburg, Germany)
Optical barcoding: A new technique to analyze tumor heterogeneity

17.00 - 17.20 **Oral Presentation IV**
Paolo Malatesta (Genoa, Italy)
Immunoescape during glioma progression

17.20 - 17.50 **Poster Session and Coffee Break**

17.50 - 19.10 **Session III**
Chair: Peter Vajkoczy

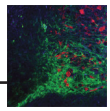
17.50 - 18.30 **Plenary Lecture III**
Frank Winkler (Heidelberg, Germany)
News from tumor microtubes in gliomas

18.30 - 18.50 **Oral Presentation V**
Pooran Singh Dewari (Edinburgh, UK)
An efficient and scalable CRISPR/Cas9 pipeline for epitope tagging in neural and glioma stem cells

18.50 - 19.10 **Oral Presentation VI**
Roland Kälin (München, Germany)
Newly identified pericyte-progenitor cells promote GBM-angiogenesis

19.15 - 20.00 **Bus Transfer to the Berlin Museum of Medical History / Charité**

20.00 **Reception at the Berlin Museum of Medical History / Charité**



Friday, May 19, 2017

9.00 - 10.20 **Session IV**
Chair: Christoph Harms

9.00 - 9.40 **Plenary Lecture IV**
Colin Watts (Cambridge, UK)
Surgical strategies to interrogate the genomics of glioblastoma

9.40 - 10.00 **Oral Presentation VII**
Hrvoje Miletic (Bergen, Norway)
Long-term prodrug administration improves lentiviral vector mediated suicide gene therapy of glioblastoma

10.00 - 10.20 **Oral Presentation VIII**
Julia Neumann (Hamburg, Germany)
Activation of shh-and wnt-signaling in neural progenitors drives formation of embryonal tumors with multilayered rosettes (ETMR)

10.20 – 10.50 **Poster Session and Coffee Break**

10.50 – 12:10 **Session V**
Chair: David Capper

10.50 – 11.30 **Plenary Lecture V**
Dolores Hambardzumyan (Altanta, USA)
Subtype specific differences in macrophage/microglia function in glioblastoma

11.30 – 11.50 **Oral Presentation IX**
Gregor Hutter (Basel, Switzerland)
CD47-Sirpa blockade induces a microglial phenotypic shift and promotes active glioblastoma phagocytosis in vivo

11.50 – 12.10 **Oral Presentation X**
Anna Gieryng (Warszawa, Poland)
Minocycline reduces production of tumor-derived osteopontin/spp1 and modulates the immune microenvironment of rat c6 gliomas

12.10 – 13.10 **Lunch (Cafeteria) and Postersession**

13.00 – 15.30 **Session VI**
Chair: Marcus Czabanka

13.10 – 13.50 **Plenary Lecture VI**
Bárbara Meléndez (Toledo, Spain)
Molecular genetics in long-term survivors of glioblastoma

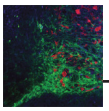
13.50 – 14.10 **Oral Presentation XI**
Claudio Giachino (Basel, Switzerland)
Opposite roles of notch signaling in the formation of distinct glioma subtypes

14.10 – 14.40 **Poster Session and Coffee Break**

14.40 – 15.20 **Plenary Lecture VII**
Richard Gilbertson (Cambridge, UK)
Mapping the origins and treatment of brain tumors

15.20 – 15.30 **Awarding of Poster Prizes: Darko Markovic**

15.30 **Departure**



List of Plenary Lectures

Cameron W. Brennan
Neurosurgery Department, Brain Tumor Center, Memorial Sloan Kettering Cancer Center, New York, USA
Molecular heterogeneity and instability of diffuse gliomas: models and clinical practice

Richard Gilbertson
Department of Oncology, Cancer Research UK Cambridge Institute, Cambridge Biomedical Campus, Cambridge, UK
Mapping the origins and treatment of brain tumors

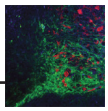
Dolores Hambardzumyan
Emory University School of Medicine, Department of Pediatrics, Atlanta GA 30322, USA
Subtype specific differences in macrophage/microglia function in glioblastoma

Eric Charles Holland
University of Washington, Fred Hutchinson Cancer Research Center, Seattle, USA
Gliomas: Big human data and mouse models

Bárbara Meléndez
Molecular Pathology Research Unit, Virgen de la Salud Hospital, Toledo, Spain
Molecular genetics in long-term survivors of glioblastoma

Colin Watts
University of Cambridge, John van Geest Centre for Brain Repair, Cambridge , UK
Surgical strategies to interrogate the genomics of glioblastoma

Frank Winkler
Neurology Clinic and National Center for Tumor Disease University Hospital Heidelberg, Clinical Cooperation Unit Neurooncology, German Cancer Research Center, 69120 Heidelberg, Germany
News from tumor microtubes in gliomas



Abstracts of Plenary Lectures

MOLECULAR HETEROGENEITY AND INSTABILITY OF DIFFUSE GLIOMAS: MODELS AND CLINICAL PRACTICE

Cameron Brennan
Neurosurgery Department, Brain Tumor Center, Memorial Sloan Kettering Cancer Center, New York, USA

Extensive genomic and proteomic analyses of diffuse gliomas have revealed a remarkable degree of structure in their mutations and gene expression. For example, signature mutations in genes, including Tert, ATRX, TP53 and chromosomal arms 1p/19q clearly distinguish typical astrocytomas and oligodendrogliomas where these entities were previously defined by histopathology. Beyond these signature genes, the constellation of other mutations is also largely constrained to a palette of well-known common events: activating alterations of receptor tyrosine kinases and NF1, and alterations targeting tumor suppressor pathways. This presentation will summarize the relationship of common mutations and currently known "omic" signatures in diffuse gliomas, and investigate some of the mechanisms and ramifications of intratumoral heterogeneity in the responses of glioblastomas to conventional and targeted therapies.

MAPPING THE ORIGINS AND TREATMENT OF BRAIN TUMOURS

Richard J. Gilbertson
Li Ka Shing Chair of Oncology, Head of Dept. of Oncology, Director, Cambridge Cancer Center, CRUK Cambridge Institute, Li Ka Shing Centre, Robinson Way Cambridge CB2 0RE, UK

Cancers are distributed unevenly across the body, but the importance of cell intrinsic factors such as stem cell function in determining organ cancer risk is unknown. Over the last 15 years we have developed the technique of cross-species genomics to map cells of origin of brain tumours in the developing nervous system. These studies have revealed that brain tumours arise from matched combinations of susceptible cell types and oncogenic mutations. More recently we have built on these data to use Cre-recombination of conditional lineage tracing, oncogene, and tumour suppressor alleles to define populations of stem and non-stem cells in multiple mouse organs and test their life-long susceptibility to tumorigenesis. We show that tumour incidence is determined by the life-long generative capacity of mutated cells. This relationship held true in the presence of multiple genotypes and regardless of developmental stage, strongly supporting the notion that stem cells dictate organ cancer risk. Using the accurate models developed through these studies, we are now conducting large preclinical studies to better identify new treatments of brain and other cancers.

GLIOMAS: BIG HUMAN DATA AND MOUSE MODELS

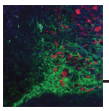
Eric Holland
Fred Hutchinson Cancer Research Center, Seattle, USA

In spite of our growing knowledge of gliomas, the clinical outcomes for this have shown little improvement. Critical issues that remain to be resolved include a better understanding of molecular heterogeneity and diagnosis that can be addressed with big data on human gliomas and mouse modeling to investigate specific genomic questions in glioma biology, and a more complete understanding of the biology of glioma immunology and response to immunotherapy using immune-competent glioma modeling systems. In addressing these issues, we have developed computational methods for displaying the landscape of gliomas based on DNA structure that divide these tumors into discrete types, and online open source tools for the research community to mine this kind of data across many tumor types. Further, mouse models of specific glioma entities provide insight into many aspects of glioma biology including components of the immune system.

MOLECULAR GENETICS IN LONG-TERM SURVIVORS OF GLIOBLASTOMA

Bárbara Meléndez
Pathology Dpt. Virgen de la Salud Hospital, Toledo 45004, Spain

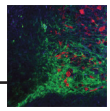
Glioblastoma (GBM) is the most common malignant adult primary brain tumor. However, despite its high lethality, a small proportion of patients have a relatively long overall survival. The identification of clinical and molecular parameters underlying this long-term survival is crucial for the design of therapies tailored to individually treat GBM patients. Several studies identified molecular biomarkers related to the long survival of GBM patients; MGMT promoter methylation predicting a better response to chemotherapy or IDH mutations conferring a better prognosis. Nevertheless, additional factors explaining this long-term survival should be present. To elucidate genetic alterations related to longer survival, we analyzed mutations and copy number variations in 30 frequently altered genes/regions in gliomas in a series of long-term survivors (LTS) of GBMs. Approximately 20% of the LTS had a mutation in the IDH1 or IDH2 genes, which was absent in non-LTS. However, alteration(s) in any component of the most frequently altered signaling pathways in GBM (RTK/PI3K, TP53 and RB1) was similarly frequent in LTS and non-LTS patients, with the only exception of PDGFRA alteration (mutation and/or amplification), more frequent in LTS. Further, a small fraction of the tumors harbored alterations in chromatin remodeling genes (ATRX, DAXX) in the absence of driver IDH or H3F3A mutations. All of these ATRX/DAXX mutated cases had particular clinical and morphological features; the presence of tumor giant cells, were younger age patients with frontal or temporal tumors and had a slightly better prognosis. A recent study suggested that ATRX alteration in animal models is related to genetic instability and that those ATRX deficient tumors may be more sensitive to therapies inducing double-stranded breaks. Therefore, future targeted therapies should take into account molecular genetic factors relevant for the long-term survival of GBM patients.



NEWS FROM TUMOR MICROTUBES IN GLIOMAS

Frank Winkler
Neurology Clinic and National Center for Tumor Disease University Hospital Heidelberg, German Cancer Research Center, 69120 Heidelberg, Germany

The recent discovery of ultra-long and thin membrane protrusions of glioma cells, called tumor microtubes (TMs), has added to our understanding of these incurable tumors. Astrocytoma (including glioblastoma) cells extend these highly functional structures to scan the brain, to invade it, to proliferate, ultimately leading to effective brain colonization. In addition, tumor cells use TMs to interconnect to one large communicating multicellular network, which includes the main tumor region, but also single tumor cells infiltrated into the normal brain tissue. We have demonstrated before that this network helps glioma cells to detect damage and repair it, and to withstand the cytotoxic effects of radiotherapy. In this talk, new data will be presented on how 1p/19q intact astrocytomas exploit neurotrophic pathways to form neurite-like extensions that give rise to functional TMs. Of note, rising evidence suggests a functional and molecular heterogeneity of TMs. Next to radiotherapy, TMs also convey resistance to surgical lesions, and TMZ chemotherapy, making them to a prime therapeutic target to tackle primary and adaptive resistance in gliomas. Unexpectedly, TMs appear also as a means for tumor cells to extensively interact with nonmalignant cells of the normal brain parenchyma. Finally, translational research will be presented that aims to target TM formation and function.



List of Oral Presentations selected from Abstracts

Dewari, Pooran Singh
MRC Centre for Regenerative Medicine, SCRM Building, The University of Edinburgh, Edinburgh, United Kingdom
AN EFFICIENT AND SCALABLE CRISPR/CAS9 PIPELINE FOR EPITOPE TAGGING IN NEURAL AND GLIOMA STEM CELLS
Dewari, P.S.; Tyrer, A.; Jacobi, A.; Behlke, M.; Pollard, S.
Keywords: CRISPR/Cas9; Glioblastoma; Epitope knock-in

Fiorelli, Roberto
Barrow Brain Tumor Research Center, Barrow Neurological Institute, Phoenix, USA
MOLECULAR AND CYTO-ARCHITECTONIC RESHAPING OF THE HUMAN SVZ DURING GLIOMA INVASION
Fiorelli, R.; Sidhu, G.; Hira, V.; Sanai, N.
Keywords: Glioma; SVZ; SDF1alpha

Friedmann-Morvinski, Dinorah
Biochemistry and Molecular Biology, Tel Aviv University, Tel Aviv, Israel
FUNCTIONAL CHARACTERIZATION OF ONCOGENIC-INDUCED CELL PASTICITY IN GLIOBLASTOMA
Friedmann-Morvinski, D; Bhargava, V.; Pilo Kerman, O.; Kenigsbuch, M.; Rousso-Noori, L.; Subramaniam, S.; Verma, I.M.
Keywords: cancer stem cells; tumor reprogramming; transcriptional network analysis

Giachino, Claudio
Department of Biomedicine, University of Basel, Basel, Switzerland
OPPOSITE ROLES OF NOTCH SIGNALING IN THE FORMATION OF DISTINCT GLIOMA SUBTYPES
Giachino, C.; Parmigiani, E.; Taylor, V.
Keywords: Glioma; Notch; Heterogeneity

Gieryng, Anna
Laboratory of Molecular Neurobiology, Nencki Institute of Experimental Biology of Polish Academy of Sciences, Warszawa, Poland
MINOCYCLINE REDUCES PRODUCTION OF TUMOR-DERIVED OSTEOPONTIN/SPP1 AND MODULATES THE IMMUNE MICROENVIRONMENT OF RAT C6 GLIOMAS
Gieryng A.; Ellert-Miklaszewska A.; Pilanc P.; Ochocka N.; Kaza B.; Kaminska B.
Keywords: GAM reprogramming; Osteopontin (Spp1); Minocycline (Mino)

Hutter, Gregor
Neurosurgery, University Hospital Basel, Basel, Switzerland
CD47-SIRPA BLOCKADE INDUCES A MICROGLIAL PHENOTYPIC SHIFT AND PROMOTES ACTIVE GLIOBLASTOMA PHAGOCYTOSIS IN VIVO.
Hutter G.; Theruvath JL.; Graef CM.; Zhang M.; Weissman IL; Mitra SS.; Cheshier SH.
Keywords: Glioblastoma; Microglia; Immunotherapies

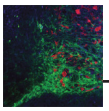
Kälin, Roland
Neurosurgical Research, LMU University Clinics, München, Germany
NEWLY IDENTIFIED PERICYTE-PROGENITOR CELLS PROMOTE GBM-ANGIOGENESIS
Kälin, R.E.; Glass, R.
Keywords: pericyte; progenitor; GBM angiogenesis

Maire, Cécile
Neurosurgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
OPTICAL BARCODING: A NEW TECHNIQUE TO ANALYZE TUMOR HETEROGENEITY
Maire, C.L.; Mohme, M.; Riecken, K.; Failla, A.V.; Helms, M.; Kolbe, K.; Fehse, B.; Westphal, M.; Lamszus, K.
Keywords: GBM; heterogeneity; barcoding

Malatesta, Paolo
DIMES, University of Genoa, Genoa, Italy
IMMUNOESCAPE DURING GLIOMA PROGRESSION
Appolloni, I.; Marubbi, D.; Alessandrini, F.; Ceresa, D.; Malatesta, P.
Keywords: PDGF-B; CD45; Gene expression profile

Miletic, Hrvoje
K. G. Jebsen Brain Tumour Research Centre, Department of Biomedicine, University of Bergen, Norway
LONG-TERM PRODRUG ADMINISTRATION IMPROVES LENTIVIRAL VECTOR MEDIATED SUICIDE GENE THERAPY OF GLIOBLASTOMA
Hossain, JA; Ystaas, LR; Talasila, KM; Ninzima, S; Riecken, K; Fehse, B; Bjerkvig, R; Miletic, H.
Keywords: glioblastoma; suicide gene therapy; EGFR

Neumann, Julia
Institute for Neuropathology, University Hospital Hamburg, Hamburg, Germany
ACTIVATION OF SHH-AND WNT-SIGNALING IN NEURAL PROGENITORS DRIVES FORMATION OF EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES (ETMR)
Neumann, J.E.; Wefers A.K.; Lambo, S.; Bianchi, E.; Bockstaller, M.; Dorostkar, M.M.; Meister, V.; Schindler P. et al.
Keywords: ETMR; Sonic hedgehog (SHH); mouse model



Abstracts of Oral Presentations

AN EFFICIENT AND SCALABLE CRISPR/CAS9 PIPELINE FOR EPITOPE TAGGING IN NEURAL AND GLIOMA STEM CELLS

Pooran Singh Dewari1, Ashley Tyrer1,2, Ashley Jacobi3, Mark Behlke3, and Steve Pollard1

1MRC Centre for Regenerative Medicine, SCRM Building, The University of Edinburgh, UK
2University of Bristol, UK
3Integrated DNA Technologies, USA

The simple design and efficacy of CRISPR-Cas9 technology has accelerated genome editing at an unprecedented scale across multiple organisms and cell types. Knock-in of small epitope tags into endogenous genes simplifies antibody-based assays, overcoming issues of specificity and sensitivity. Here, we demonstrate highly efficient and scalable CRISPR/Cas9-assisted epitope knock-in using mouse and human primary neural stem (NS) cells and glioblastoma tumour-derived cultures. Three different methods of Cas9 delivery were tested: 1) transient expression through a plasmid, 2) recombinant Cas9 (rCas9) protein transfection, and 3) constitutive expression of Cas9 in cells. We find that the rCas9 protein delivery facilitates efficient knockin of V5 tag (5-10%) without requirements for any selection strategy. Delivery of ribonucleoprotein complexes containing synthetic dual-guide RNA (crRNA 36-mer and tracrRNAs 67-mer) provided even further gains in efficiency, with knock-in efficiencies increase to 5-30% depending on target genes. Similar efficiencies were achieved in mouse and human NS and glioma stem cells. Importantly, with these optimized conditions and a newly developed web-based tool for crRNA and donor DNA design, we were able to demonstrate medium throughput epitope tagging in a 96-well plate format. 192 transcription factors (key regulators of neural stem cell self-renewal and differentiation) were tested for tagging in parallel, and 60 of these were effectively tagged with V5. Our method provides a step-change in our ability to interrogate mammalian proteins in stem cells and their glioma counterparts. As a proof-of –principle, we used the newly tagged glioma cell lines for Sox3-V5 and performed ChIP-SICAP (selective isolation of chromatin associated proteins). In summary we have developed a highly efficient and scalable pipeline for tagging of endogenous proteins in mouse and human neural and glioma stem cells.

MOLECULAR AND CYTO-ARCHITECTONIC RESHAPING OF THE HUMAN SVZ DURING GLIOMA INVASION

Fiorelli R.; Sidhu G.; Hira V.; Sanai N.

Dep. of Neurosurgery, Barrow Brain Tumor Research Center, Barrow Neurological Institute, Phoenix, Arizona

The sub-ventricular zone (SVZ) lining the ventricles is a specialized niche for Neural Stem Cells (NSCs). Clinical studies have shown that Glioma patients with tumors contacting the SVZ have earlier recurrences and overall worse prognosis than non-SVZ-contacting tumors. Molecular and cellular events regulating Glioma homing to the SVZ are largely unknown.

Here, we have compared normal (N=5) and high-grade Glioma samples (N=20) of the human SVZ by Immunofluorescence and volumetric image analysis. Glioma samples showed gradual stages of invasion; SVZ cytoarchitecture varied from relatively normal (low-invasion) to completely disrupted (high-invasion). Stainings for Laminin and Fibronectin revealed upregulation of vascular-associated extracellular fractones, size and density of which increased up to 3 fold in low-invasion Glioma samples compared to controls. In contrast, high-invasion samples showed disappearance of the fractones network in favor of local neo-vascularization.

Analysis of the normal SVZ by qPCR revealed local enrichment of the chemokine SDF1α, ligand for the CXCR4 receptor and potent in vitro chemoattractor of Glioma Stem Cells. Histology revealed SDF1α expression specifically in the ependymal layer. CXCR4 was enriched in the NSCs-astrocyte ribbon, as well as present in Microglia. In low-invasion Glioma samples, ependymal showed a 3-fold upregulation of SDF1α. Density of Microglia was proportional to SDF1α expression. Accordingly, CXCR4 expression was widely increased in the neighboring parenchyma, including in the tumor cell population. Our study highlights an active reshaping of the ependymal, and the hypocellular layers of the human SVZ during Glioma invasion. Further, our data indicate that the SDF1α-CXCR4 signaling axis is involved in Glioma homing to the human SVZ, supported by previous observations in rodent models.

FUNCTIONAL CHARACTERIZATION OF ONCOGENIC-INDUCED CELL PLASTICITY IN GLIOBLASTOMA

Dinorah Friedmann-Morvinski1,2, Vipul Bhargava4, Ori Pilo Kerman1, Mor Kenigsbuch1, Liat Rousso-Noori1, Shankar Subramaniam4, Inder M. Verma3

1Department of Biochemistry and Molecular Biology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel
2Sagol School of Neuroscience, Tel Aviv University, Tel Aviv.
3The Salk Institute for Biological Studies, La Jolla, USA
4Department of Bioengineering, University of California at San Diego, La Jolla, California 92093, USA

Glioblastoma (GBM) is the most common and lethal form of intracranial tumor. Using a Cre-inducible lentiviral GBM mouse model we recently showed that gliomas can originate from terminally differentiated neurons and astrocytes, which can dedifferentiate to a stem cell-like state upon transformation. In this study, we performed whole transcriptome analysis and confirmed that transformed dedifferentiated astrocytes and neurons acquired a stem/progenitor cell state, although they still retained gene expression memory from their parental cell. Functional analysis of the transcriptomics data revealed involvement of the Wnt signaling, cell cycle and the focal adhesion pathways in defining the state of the dedifferentiated cell-types. Our analysis further revealed conservation of a gene interaction network in both dedifferentiated cell-types. This network exhibited a modular architecture, connecting components of the cell cycle pathway to Wnt signaling and the focal adhesion pathways, with the gene Spp1, also known as osteopontin (OPN) serving as a key common node connecting these three pathways. Specific inhibition of OPN in both murine and human glioma tumors prolonged mice survival. We are currently validating the expression of additional interacting partners. We believe that genetic perturbation of key players and/or the abolishment of their interactions can help elucidate the regulatory mechanism of this network in maintaining the dedifferentiated state of the transformed neurons and astrocytes.

OPPOSITE ROLES OF NOTCH SIGNALING IN THE FORMATION OF DISTINCT GLIOMA SUBTYPES

Giachino, C.; Parmigiani, E.; Taylor, V.

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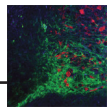
Neural stem cells in the postnatal brain are believed to be one origin of brain tumors such as gliomas. The Notch signaling pathway is required for neural stem cell maintenance and, accordingly, promotes a self-renewing stem cell-like state in glioma cells. Therefore, Notch signaling is believed to be oncogenic in glioma, primarily by virtue of its stem cell promoting activity. However, inactivating mutations in Notch pathway components and low Notch signaling activity have been identified in glioma subtypes CD47-Sirpa blockade induces



in humans, suggesting a tumor suppressive role. We addressed the role of Notch signaling in glioma formation using conditional genetics and lineage tracing in mouse models of human brain tumors and discovered a context dependent function for the Notch pathway in distinct glioma subtypes. We found that gliomas driven by either p53 loss of function or Akt gain of function can originate from a cell population with high Notch signaling activity. However, surprisingly, Notch has opposite functions in these glioma subtypes. Genetic deletion of core Notch pathway components accelerates growth of gliomas driven by p53 loss of function and, conversely, genetic activation of the Notch pathway reduces glioma formation. In stark contrast, genetic deletion of Notch pathway components delays the development of gliomas driven by gain of Akt signaling. Interestingly, individual Notch receptors have distinct functions during glioma development, and only specific Notch receptors or receptor combinations can activate a tumor suppressive signal. Hence, Notch receptor paralog and glioma subtype dictate the tumor suppressive versus oncogenic role of Notch signaling.

MINOCYCLINE REDUCES PRODUCTION OF TUMOR-DERIVED OSTEOPONTIN/SPP1 AND MODULATES THE IMMUNE MICROENVIRONMENT OF RAT C6 GLIOMAS

Gieryng A.; Ellert-Miklaszewska A.; Pilanc P.; Ochocka N.; Kaza B.; Kaminska B.

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Glioma associated microglia and macrophages (GAMs) support tumor invasion and contribute to immunosuppression. We demonstrated a critical role of Osteopontin (Spp1), a potent immune cell attractant and activator, secreted by glioma cells in microglia reprogramming and glioma progression. Minocycline (Mino) has been reported to affect glioma growth via the inhibitory effect on matrix metalloproteinases in microglia or induction of autophagy in glioma cells. We hypothesized that Mino may impair Spp1 expression in glioma cells that would affect responses of immune cells in a tumor microenvironment. We demonstrate that Mino inhibits Spp1 expression in cultured glioma cells. Systemic application of Mino (30 mg/kg b.w) to animals implanted intracranially with C6-Luc+ glioma cells reduced tumor volumes at day 14th as determined using an In Vivo-Imaging Xtreme. Immune heterogeneity of glioma microenvironment was analysed by FACS in controls and Mino treated animals. Expression of selected GAM markers in sorted CD11b+ cells from glioma-bearing hemispheres and cytokine production in glioma-bearing hemispheres were measured. Mino treatment did not affect microglia accumulation, but blocks protumorigenic activation in generated CD11b+ cells and increase expression of selected GAM markers. The increased accumulation of macrophages and leukocyte subpopulations was detected in Mino-treated rats. Profiles of cytokine production in tumor-bearing hemispheres from Mino-treated animals showed differences in the production of pro-inflammatory cytokines and macrophage attractants when compared with controls. We conclude that Mino treatment reduced the expression of pro-invasive factors secreted by glioma cells (i.e. Spp1) that resulted in immunosuppression switch and the increased expression of antitumor response genes in glioma-bearing hemispheres and GAMs. Our results validate Mino treatment as a promising strategy to block Spp1 production and GAM reprogramming.

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A MICROGLIAL PHENOTYPIC SHIFT AND PROMOTES ACTIVE GLIOBLASTOMA PHAGOCYTOSIS IN VIVO.

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Modulation of tumor-associated macrophages and microglia (TAMs) in glioblastoma (GBM) presents as a promising anti-tumor strategy. However, little is known about the phenotypic organization of the TAM-pool and the role of GBM subtypes in shaping their specific immunologic environment. Therefore, we sought to model the composition of microglia and peripheral macrophages within the GBM-TAM-pool. Further, we aimed at assessing the response of the TAM pool to an important macrophage-modulating therapy, CD47-Sirp alpha blockade. Using orthotopically xenografted, immunodeficient mice and syngeneic mouse models with genetically color-coded macrophages (Ccr2RFP) and microglia (Cx3cr1GFP), we found the TAM composition to be dependent on the rate of tumor growth. While microglia dominated TAMs in slow growing tumors, high passaged cell lines induced a mixed pool predominantly composed of microglia. Blockade of the "don't eat me" signal CD47 by anti-CD47 antibodies prompted macrophage and microglial-induced phagocytosis in vivo and lead to a marked microglial morphology change assessed by intracranial in vivo imaging. The therapeutic efficacy of anti-CD47 treatment was preserved in case of Ccr2-disruption and deficient macrophage recruitment to the brain. Anti-CD47 induced microglial phagocytosis alone was able to reduce tumor burden. Under anti-CD47 treatment, macrophages changed their transcriptional profile towards a more pro-inflammatory and M1-polarized signature whereas microglia upregulated phagocytic effector genes but was devoid of an inflammatory response. These results emphasize the importance of both resident microglia and invading macrophages in GBM biology. Moreover, CD47-Sirp alpha disruption caused an important phenotypic and functional status change of resident microglia, which will have implications for central nervous system (CNS) immunotherapies in the future.

NEWLY IDENTIFIED PERICYTE-PROGENITOR CELLS PROMOTE GBM-ANGIOGENESIS

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2Walter-Brendel-Centre of Experimental Medicine, Ludwig-Maximilians University Munich, Germany

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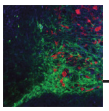
4Department of Neurosurgery, University Hospital Center Schleswig Holstein, Kiel, Germany.

Pericytes control angiogenesis as well as blood-brain barrier integrity in the developing CNS and in gliomas. Pericytes were postulated to derive from mitotically active, mature pericytes, which can be identified by the expression of markers including PDGFR-B, NG2, desmin or CD146. Using a newly established lineage-tracing mouse-model we show that mature pericytes largely originate from marker-negative cells. In this model stable fluorescence-reporter activity was obtained after tamoxifen (TAM) inducible genetic recombination



controlled by a modified nestin-promoter element (NES-creER2). To study neo-angiogenesis in the adult brain we orthotopically inoculated syngeneic glioma cells and observed angiogenesis as well as pericyte-expansion over time. A smaller number of fluorescent cells was detected at early tumourigenesis (7 days after tumour implantation; P7), these had an amoeboid-like morphology and were located distant from the vasculature. At P14 and P21, when larger and strongly angiogenic tumours had formed, the progeny of traced cells had strongly expanded, was closely associated with the endothelia and exhibited an elongated cell-shape. Strikingly, the vast majority of recombined cells (78%) was negative for all pericyte-markers at P7, but acquired pericyte-markers (81%) at P21 – suggesting that mature pericytes derive from undifferentiated progenitors. These pericyte progenitors are not restricted to pathological angiogenesis, but were also observed in the developing CNS. Lineage-tracing experiments in bone-marrow chimeric glioma models indicated that pericyte progenitors are endogenous to the brain. Targeting pericyte progenitors in lineage ablation studies efficiently reduced the number of recombined cells, diminished the tumoural vascular network and decreased the tumour-burden by 50%. Our data indicate that newly generated pericytes origin from a previously unrecognised, brain-resident pericyte progenitor which can provide an excellent target for new anti-angiogenic treatments.

OPTICAL BARCODING: A NEW TECHNIQUE TO ANALYZE TUMOR HETEROGENEITY

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Neurosurgery, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Most of the genomic and epigenetic alterations in glioblastoma (GBM) have been described thanks to the effort to the Tumor Cancer Genome Atlas (TCGA) and others, highlighting the highly heterogeneous and complex genomic landscape of GBM. Our understanding of how such heterogeneity is maintained is still unclear, but tumor growth is not a linear process and it is shaped by the interaction with the microenvironment such as blood vessels and immune cells. Driven by selective pressure, the tumor follows a clonal evolution model that leads to the emergence of new subpopulation and regional heterogeneity. To develop more successful therapies we need to understand the heterogeneity of GBM at the single cell level in order to eliminate the emergence of drug resistant clones. We generated a new in vivo and in vitro model that maintains the cellular heterogeneity of the original tumor by establishing GBM-patient derived cell lines (PDCL) and mouse xenograft (PDX) from tissue resections. To be able to follow the growth progression of multiple clones in vivo we took advantage of an optical barcoding system to track and quantify clonal evolution. Using a combination of 6 different color-coded lentiviral vectors we isolated single cells out of GBM-PDCL and tagged them with 21 different color combinations. These clones were then grown separately or as a mix in vitro and in vivo after intracranial injection. Using this innovative technique, we tracked the fate of individual clones and analyzed their growth dynamics in different microenvironments. We discovered that fast growing clones in vitro are not necessarily the ones that survive and are responsible for most of the tumor growth in vivo. Interestingly, these clones that do not have the capacity to overgrow other clones when injected in the brain as a mix, are still tumorigenic when injected alone. Moreover, we discovered that some clones are more resistant to radiotherapy and standard of care treatment (irradiation plus temozolomide) than others, opening the possibility to identify specific clonal subpopulations in the tumor that are resistant to treatment and will probably develop into the major resistant clone when the tumor will recur.

Understanding how the tumor heterogeneity is shaped by the microenvironment and how the different clonal population react to treatment will help predict drug treatment efficiency and potential resistance.

IMMUNOESCAPE DURING GLIOMA PROGRESSION

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1 IRCCS AOU S. Martino-IST Genova, Italy

2 DIMES - Università degli studi di Genova, Italy

Glioma is a primary brain tumor whose grade IV, glioblastoma, is the most frequent and incurable form. To study the mechanisms involved in glioma progression, we generated a mouse model of glioma by overexpressing the PDGF-B oncogene in embryonic neural progenitor cells in vivo. We demonstrated that all the animals develop tumors that initially show a low-grade phenotype and do not form new tumors when transplanted in syngeneic adult animals. However, these tumors invariably undergo a malignant progression towards a high-grade phenotype with tumor-propagating potential in vivo. A microarray analysis on these tumors revealed a strong downregulation of immune-stimulatory genes during malignant progression. Moreover, immunistochemical and citofluorimetric assays showed that high-grade tumors have a lower immune infiltration of CD45 and CD8 positive cells. These data suggest that an important step during glioma progression is the acquisition of the ability to hide from the immune system thus preventing an immune response. More strikingly, we noticed that immunodeficient mice represent a permissive background for low-grade gliomas transplantation and progression. Cells obtained from low-grade tumors generate secondary gliomas in Nod/Scid mice and, surprisingly, acquire the ability to graft in immunocompetent mice. All these data strongly suggest a cross-talk between glioma and immune cells that regulate glioma formation and progression.

LONG-TERM PRODRUG ADMINISTRATION IMPROVES LENTIVIRAL VECTOR MEDIATED SUICIDE GENE THERAPY

Jubayer A Hossain1,2,, Lars Rømo Ystaas1, Krishna M Talasila1, Sandra Ninzima1, Kristoffer Riecken3, Boris Fehse3, Rolf Bjerkvig1,4 and Hrvoje Miletic1,2

1K. G. Jebsen Brain Tumour Research Centre, Department of Biomedicine, University of Bergen, Norway

2 Department of Pathology, Haukeland University Hospital, Norway

3 Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany.

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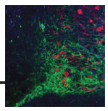
We have previously shown that lentiviral vector mediated herpes simplex virus thymidine kinase (HSV-Tk)/ ganciclovir (GCV) therapy is a very promising therapeutic option for the treatment of glioblastoma (GBM). Although this therapy leads to complete remission of GBM in an orthotopic PDX model, recurrent tumors are observed which contain a fraction of Tk-GFP+ cells surviving 3-4 weeks of prodrug administration. We sorted Tk-GFP+ glioma cells from recurrent tumors and observed that the cells are less proliferative and retain sensitivity to GCV. Thus, we showed that short-term prodrug delivery- used in clinical gene therapy trials - fails to eliminate a fraction of glioma cells, which are slow proliferating; we hypothesized that a longer period of prodrug administration would provide an enhanced therapeutic effect. As long-term prodrug we used valganciclovir (valGCV), which is similar to GCV, but tailored for oral administration. Prolonged administration of valganciclovir (valGCV) resulted in a significant survival advantage compared to short-term (3 weeks) GCV application. Nonetheless, the majority



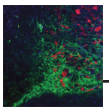
of animals treated with valGCV also developed recurrent tumors. These tumors were more invasive compared to the primary tumors and showed significant upregulation of the epidermal growth factor receptor (EGFR). We are currently investigating signaling pathways upstream and downstream of EGFR. Our results warrant a treatment combination of Tk/ValGCV gene therapy with EGFR inhibitors, which we are currently investigating.

SIMULTANEOUS ACTIVATION OF SHH- AND WNT-SIGNALING IN NEURAL PROGENITORS DRIVES FORMATION OF EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES (ETMR) AND INDICATES POTENTIAL THERAPEUTIC AVENUES

Julia E. Neumann, Annika K. Wefers, Sander Lambo, Edoardo Bianchi, Marie Bockstaller, Mario M. Dorostkar, Valerie Meister, Pia Schindler , Andrey Korshunov, Katja von Hoff , Johannes Nowak, Monika Warmuth-Metz, Marlon R. Schneider, Ingrid Müller-Renner, Daniel J. Merk , Mehdi Shakarami, Rainer Glass, Jennifer A. Chan, M. Mark Taketo, Philipp Neumann, Marcel Kool and Ulrich Schüller

Institute for Neuropathology, University Hospital Hamburg, Hamburg, Germany

Embryonal Tumors with Multilayered Rosettes (ETMRs) have recently been described as a new entity of rare pediatric brain tumors with fatal outcome. We show here that the overexpression of Lin28A, which is a hallmark of human ETMRs, augments Sonic Hedgehog (Shh)- and Wnt-signaling through the regulation of let7-miRNAs. The simultaneous activation of Shh- and Wnt-signaling in turn is sufficient to induce ETMRs from neural precursors of the murine cortical subventricular zone (SVZ). These tumors are well responsive to the Shh-inhibitor Arsenic trioxide (ATO), and the treatment of immunocompromised mice with orthotopically injected human ETMR-cells finally demonstrates that inhibition of Shh-signaling may serve as a therapeutic option for patients with ETMRs.



List of Poster Presentations

(The number of the poster presentation corresponds to the numbers on the poster boards. Please hang your poster there.)

1. Adamski, Vivian
Neurosurgery, UKSH Kiel, Arnold-Heller-Str.3, No.41, 24105 Kiel, , Vivian.Adamski@uksh.de
"REVERSE SIGNALING" OF THE TRANSMEMBRANE CHEMOKINE CXCL16 IN GLIOBLASTOMAS
Adamski, V.; Mentlein, R.; Lucius, R.; Synowitz, M.; Held-Feindt, J.; Hattermann, K.
Keywords: Dormancy; glioblastoma; stemness

2. Adamski, Vivian
Neurosurgery, UKSH Kiel, Arnold-Heller-Str.3, No.41, 24105 Kiel, Vivian.Adamski@uksh.de
DORMANT HUMAN GLIOBLASTOMA CELLS OWN STEM CELL CHARACTERISTICS AND ARE DISTINCTLY AFFECTED BY CHEMOTHERAPEUTIC TREATMENT STRATEGY
Adamski, V.; Hempelmann, A.; Flöh, C.; Lucius, R.; Synowitz, M.; Hattermann, K.; Held-Feindt, J.
Keywords: Dormancy; glioblastoma; stemness

3. Alessandrini, Francesco
DIMES, University of Genoa, Largo Rosanna Benzi 10, 16132 Genoa, Italy, francescoalessandrini89@gmail.com
COMMON FEATURES BETWEEN EGFRVIII AND PDGF-B INDUCED GLIOMAS MODELS
Alessandrini, F.; Ceresa, D.; Appolloni, I.; Marubbi, D.; Malatesta, P.
Keywords: Oligodendrocyte precursors; RNAseq; glioblastoma

4. Barciszewska, Anna-Maria
Chair and Clinic of Neurosurgery and Neurotraumatology, Karol Marcinkowski University of Medical Sciences, Przybyszewskiego 49, 60-355 Poznan, Poland, abarcisz@man.poznan.pl
TENASCIN-C AS A THERAPEUTIC TARGET FOR HIGH GRADE GLIOMAS
Barciszewska, A-M; Wawrzyniak, D; Piwecka, M; Szpakowska, M; Nowak, S; Rolle K.
Keywords: tenascin-C; high grade gliomas; RNAi

5. Barta, Aliz
Department of Biophysics and Cell Biology, University of Debrecen, Egyetem tér 1, 4032 Debrecen, Hungary, aliz942@gmail.com
SYNTHETIC FLAVONOID COMPOUNDS INHIBIT GLIOBLASTOMA CELL PROLIFERATION AND VIABILITY
Barta, A.; Pethö, Z.; Pajtás, D.; Varga, Z.
Keywords: Glioblastoma; Falvonoid; Cell proliferation

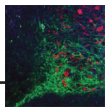
6. Bastiancich, Chiara
Micro et Nanom,decines Translationnelles - INSERM UMR-S 1066 / CNRS 6021, Universit, d'Angers, 4 Rue Larrey, 49933 Angers, France, chiara.bastiancich@uclouvain.be
LAUROYL-GEMCITABINE LIPID NANOCAPSULES AS MULTI-DRUG COMBINATION NANOMEDICINE HYDROGEL FOR THE LOCAL TREATMENT OF GLIOBLASTOMA
Bastiancich, C.; Bianco, J.; Lemaire L.; Danhier F.; Préat V.; Bastiat G.; Lagarce F.
Keywords: Glioblastoma; Hydrogels; Nanomedicine

7. Behnan, Jinan
Immunology, The Norwegian Center for Stem Cell Research, Institute of Clinical Medicine, Sognsvannsveien 9, 0317 Oslo, Norway, jinan_bahnan@hotmail.com
DIFFERENTIAL PROPAGATION OF STROMA AND CANCER STEM CELLS DICTATES TUMORIGENESIS AND MULTIPOTENCY
Jinan Behnan; Biljana Stangeland; S. A. Mohieb Hosainey; Mrinal Joel; Joel C. Glover; Pauline Iskason; Jan Brinchmann
Keywords: Tumor stroma; Cancer stem cells; Glioblastoma subtyping

8. Buonfiglioli, Alice
Cellular Neurosciences, Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Str. 10, 13092 Berlin, alice.buonfiglioli@mdc-berlin.de
THE ROLE OF LET-7 MICRORNAS AS ACTIVATORS OF GLIOMA-ASSOCIATED MICROGLIA/MACROPHAGES
Buonfiglioli, A.; Wolf, S.; Kettenmann, H.; Lehnardt, S.
Keywords: microRNAs; microglia; glioblastoma

9. Busek, Petr
Inst of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, U nemocnice 5, 12853 Prague 2, Czech Republic, busekpetr@seznam.cz
CELLS WITH CHARACTERISTICS OF CANCER ASSOCIATED FIBROBLASTS IN GBM AND THEIR EFFECT ON MIGRATION OF ENDOTHELIAL AND GLIOMA CELLS
Keywords: cancer-associated fibroblastas, seprase, migration

10. Cantero Montenegro, Diana
Department of Pathology Neuropathology, 12 de Octubre University Hospital, Avda de Cordoba s/n, 28041 Madrid, Spain, diana_cantero@h12o.es
FREQUENT CHROMATIN REMODELING ATRX AND DAXX GENE ALTERATIONS IN ABSENCE OF DRIVER IDH OR H3.3 MUTATIONS IN GIANT CELL GLIOBLASTOMAS
Cantero,D;Nicky,DH;Rodriguez de Lope,A;De Nerve,N;Gutierrez,MJ;Le Mercier,M;Mollejo,M;Salmon,I;Hernandezain,A;Melendez,B
Keywords: Giant Cell Glioblastoma; Next-Generation Sequencing; chromatin remodeling gene



11. Capper, David
Neuropathology, Charite Berlin, Virchowweg 15, 10117 Berlin, , dacapper@yahoo.com
DNA METHYLATION-BASED CLASSIFICATION OF HUMAN CENTRAL NERVOUS SYSTEM TUMORS
David Capper; David T.W. Jones; Martin Sill;, Volker Hovestadt; Daniel Schrimpf; Damian Stichel; Dominik Sturm; Christian Koelsch, Andreas von Deimling, Stefan Pfister
Keywords: methylation; DNA; classification

12. Ceresa, Davide
DIMES, University of Genoa, Largo Rosanna Benzi 10, 16132 Genoa, Italy, davide.ceresa91@gmail.com
TRACKING GLIOMA PROGRESSION BY GENETIC BARCODING
Ceresa, D.; Alessandrini, F.; Appolloni, I.; Ceccherini, I.; Caroli, F.; Malatesta P.
Keywords: NGS; PDGF-B; Tumor-heterogeneity

13. Chen, Zhihong
Pediatrics, Emory University, 1760 Haygood Dr., HSRB E-376, 30329 Atlanta, United States, zhihong.chen@emory.edu
CELLULAR AND MOLECULAR IDENTITY OF TUMOR-ASSOCIATED MACROPHAGES IN GLIOBLASTOMA
Chen, Z.; Feng, X.; Herting, C.; Alvarez Garcia, V.; Nie, K.; Pong, W.; Rasmussen, R.; Dwivedi B.; Seby, S.; Wolf, S.; Gutmann, D.; Hambardzumyan, D.
Keywords: malignant glioma; microglia; inflammatory monocytes

14. Ciechomska, Iwona
Neurobiology Center, Nencki Institute of Experimental Biology, 3 Pasteur, 02-093 Warzaw, Poland, i.ciechomska@nencki.gov.pl
EXPLORING GLIOMA HETEROGENEITY USING IMMUNOBLOTTING AND IMMUNOCYTOCHEMISTRY
Iwona A. Ciechomska, Kamil Wojnicki, Ryszard Czepko, Mariusz Banach, Bartosz Czapski, Pawel Nauman, Katarzyna Kotulska, Wieslawa Grajkowska, Marcin Roszkowski, Tomasz Czernicki, Andrzej Marchel, Bozena Kaminska
Keywords: glioma; heterogeneity; immunochemistry

15. Dedobbeleer, Matthias
GiGA Neurosciences, University Liége, Avenue Hippocrate 15, 4000 Liége, Belgium, mdedobbeleer@ulg.ac.be
RADIOPROTECTIVE ROLE OF MKP-1 IN GLIOBLASTOMA CELLS IN RESPONSE TO A CXCL12-STIMULATION PRODUCED IN THE SUBVENTRICULAR ZONES
Dedobbeleer M.; Willems E.; Lombard A.; Digregorio M.; Lumapat P.; DiValentin E.; Goffart N.; Rogister B.
Keywords: Glioblastoma; Phosphatase; Radiotherapy

16. Deshors, Pauline
team 11, CRCT, INSERM, 2 Avenue Hubert Curien, 31100 Toulouse, France, pdeshors@laposte.net
RADIO-INDUCED TRANSDIFFERENTIATION OF GLIOBLASTOMA STEM CELLS INTO ENDOTHELIAL CELLS
Deshors, P.; Malric, L.; Arnauduc, F.; Lemari,, A.; Cohen-Jonathan-Moyal, E.; Toulas, C.; Courtade-Saidi, M.; Evrard, S.M.
Keywords: Glioblastoma stem cells; Ionizing radiation; Glio-endothelial Transdifferentation

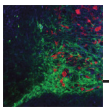
17. Dewari, Pooran Singh
Dr Pooran Singh Dewari, MRC Centre for Regenerative Medicine, SCRM Building, The University of Edinburgh, 5 Little France Drive, EH16 4UU Edinburgh, UK, pooran.dewari@ed.ac.uk
AN EFFICIENT AND SCALABLE CRISPR/CAS9 PIPELINE FOR EPITOPE TAGGING IN NEURAL AND GLIOMA STEM CELLS
Pooran Singh Dewari, Ashley Tyrer, Ashley Jacobi, Mark Behlke, Steve Pollard
Keywords: CRISPR/Cas9; glioblastoma; Epitope knock-in

18. Di Tacchio, Mariangela
Institute of Neurology Edinger Institute, Medical School Goethe University Frankfurt, Heinrich-Hoffmann-Str. 7, 60528 Frankfurt am Main, Mariangela.DiTacchio@kgu.de
ENDOTHELIAL CELL-DERIVED ANGIOPOIETIN-2 IS A THERAPEUTIC TARGET IN TREATMENT-NAIVE AND BEVACIZUMAB-RESISTANT GLIOBLASTOMA
Di Tacchio, M.; Scholz, A.; Harter, P. N.; Cremer, S.; Yalcin, B. H.; Gurnik, S.; Plate K. H.; Reiss, Y.
Keywords: anti-angiogenic therapy; glioblastoma; macrophage polarization

19. Dietterle, Johannes
Neurosurgery, University Hospital Leipzig, Oststraße 62, 04317 Leipzig, johannes.dietterle@medizin.uni-leipzig.de
CARNOSINE INHIBITS THE INFILTRATIVE GROWTH OF GLIOBLASTOMA CELLS
Dietterle J.; Oppermann H.; Meixensberger J.; Gaunitz F.
Keywords: Glioblastoma multiforme; Carnosine; Co-culture

20. Digregorio, Marina
Giga neurosciences, Université de Liége Ulg, B34 Quartier Hôpital Avenue de l'Hôpital, 11, 4000 Liége, Belgique, marina.digregorio@alumni.ulg.ac.be
TUMOUR MASS VERSUS SUBVENTRICULAR ZONE: MOLECULAR AND FUNCTIONAL CHARACTERISATION
Digregorio, M.; Willems, E.; Dedobbeleer, M.; Rogister, B.; Scholtes, F.
Keywords: Glioblastoma; Recurrences; Subventricular zone

21. Duman, Ceren
Clinical Neurobiology, German Cancer Research Center DKFZ, Im Neuenheimer Feld 280, 69120 Heidelberg, c.duman@dkfz.de
ROLE OF ACYL-COA BINDING PROTEIN ACBP IN GLIOBLASTOMA MULTIFORME TUMOR INITIATION AND DEVELOPMENT
Ceren Duman; Angelika Hoffmann; Martin Bendszus; Julieta Alfonso
Keywords: metabolism; fatty acid; proliferation



22. Eisemann, Tanja
Signal Transduction and Growth Control, DKFZ, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany, t.eisemann@dkfz.de
THE MUCIN-LIKE GLYCOPROTEIN PODOPLANIN IN GLIOBLASTOMA
Tanja Eisemann, Barbara Costa, Ana Martín-Villalba, Michel Mittelbronn, Peter Angel, Heike Peterziel
Keywords: Glioma; Tumor microenvironment; Podoplanin

23. Ellert-Miklaszewska, Aleksandra
Laboratory of Molecular Neurobiology, The Nencki Institute of Experimental Biology of the Polish Academy of Sciences, 3 Pasteur str, 02-093 Warsaw, Poland, a.ellert@nencki.gov.pl
EXPRESSION AND FUNCTION OF NFAT NUCLEAR FACTOR OF ACTIVATED T CELLS TRANSCRIPTION FACTORS IN HUMAN GLIOMAS
Ellert-Miklaszewska, A.; Wojtas, B.; Gielniewski, B.; Ochocka, N.; Maleszewska, M.; Krol, S.; Wojnicki, K.; Kaminska, B.
Keywords: NFAT; glioma

24. Feldheim, Jonas
Department of Neurosurgery, University of Würzburg, Josef-Schneider-Str. 11, 97080 Würzburg, jonas.feldheim@googlemail.com
ACTIVATING TRANSCRIPTION FACTOR 5: A NEW THERAPEUTIC TARGET IN ASTROCYTOMAS OF DIFFERENT WHO-GRADES AND VARYING BIOLOGICAL BEHAVIOUR
Feldheim, J.; Kessler, A. F.; Schmitt, D.; Wilczek, L.; Linsenmann, T.; Ernestus, R.-I.; Hagemann, C.; Löhr, M.
Keywords: Glioblastoma; Astrocytoma; ATF5

25. Fiorelli, Roberto
Barrow Brain Tumor Research Center, Barrow Neurological Institute, 350 W Thomas Rd, 85013 Phoenix, USA, roberto.fiorelli@dignityhealth.org
MOLECULAR AND CYTO-ARCHITECTONIC RESHAPING OF THE HUMAN SVZ DURING GLIOMA INVASION
Fiorelli R.; Sidhu G.; Hira V.; Sanai N.
Keywords: glioma, SVZ, SDF1alpha

26. Flüh, Charlotte
Department of Neurosurgery, University Hospital of Schleswig-Holstein, Campus Kiel, Arnold-Heller-Strasse 3, Haus 41, 24105 Kiel, charlotte.flueh@uksh.de
NKG2D LIGANDS IN GLIOMA STEM-LIKE CELLS: EXPRESSION IN SITU AND IN VITRO
Flüh, C; Chitadze, G; Adamski, V; Hattermann, K; Synowitz, M; Kabelitz, D; Held-Feindt, J.
Keywords: glioblastoma; NKG2DL; stem cells

27. Friedmann-Morvinski, Dinorah
Biochemistry and Molecular Biology, Tel Aviv University, Tel Aviv University, 69978 Tel Aviv, Israel, dino@post.tau.ac.il
FUNCTIONAL CHARACTERIZATION OF ONCOGENIC-INDUCED CELL PASTICITY IN GLIOBLASTOMA
Dinorah Friedmann-Morvinski, Vipul Bhargava, Ori Pilo Kerman, Mor Kenigsbuch, Liat Rousso-Noori, Shankar Subramaniam, Inder M. Verma
Keywords: cancer stem cells; tumor reprogramming; transcriptional network analysis

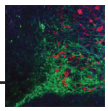
28. Gavard, Julie
CRCINA, Team SOAP, Universite Nantes, CNRS, INSERM, 8 quai Moncousu, 44000 Nantes, France, julie.gavard@inserm.fr
APELIN FUNCTION IN ENDOTHELIAL/TUMOR INTERACTION IN GLIOBLASTOMA
Harford-Wright, E; Andre-Gregoire, G; Jacobs, K; Treps, L; Davenport, AP; Glen, RC; Bidere, N; Gavard, J.
Keywords: glioblastoma stem cell; vascular niche; apelin

29. Geiss, Carsten
Johann-Joachim-Becher-Weg 15, 55128 Mainz, Germany, cargeiss@uni-mainz.de
GLUTAMATE METABOLISM AND INFLAMMATORY PROFILE IN TUMOR ASSOCIATED MICROGLIA/MACROPHAGES
Geiss, C.; Savaskan, N.; R,gnier-Vigouroux, A.
Keywords: glutamate metabolism; glioma-associated microglia/macrophages; inflammatory status

30. Gerigk, Magda
Department of Engineering, University of Cambridge, Trumpington Street, CB2 1PZ Cambridge, UK, mg780@cam.ac.uk
A MICROFLUIDIC SYSTEM FOR GBM PROGRESSION STUDIES
Gerigk, M.; Tönisen, F.; Cullen, C.; Huang, Y.Y.S.
Keywords: Microfluidics; Cancer progression; Tumor microenvironment

31. Giachino, Claudio
Department of Biomedicine, University of Basel, Mattenstrasse 28, 4058 Basel, Switzerland, claudio.giachino@unibas.ch
OPPOSITE ROLES OF NOTCH SIGNALING IN THE FORMATION OF DISTINCT GLIOMA SUBTYPES
Giachino, C.; Parmigiani, E.; Taylor, V.
Keywords: glioma; Notch; heterogeneity

32. Gielniewski, Bartlomiej
Nencki Institute of Experimental Biology, Pasteura 3, 02-093 Warsaw, Poland, b.gielniewski@nencki.gov.pl
THE IMPACT OF IDH1/2 MUTATIONS ON SOCS GENE METHYLATION AND DEREGULATION OF STAT SIGNALING IN GLIOMAS
Gielniewski, B.; Wojtas, B.; Maleszewska, M.; Krol, S. K.; Ciechomska, I.; Kaminska, B.
Keywords: IDH1/2 mutation; SOCS gene methylation; STAT signaling



33. Gieryng, Anna
Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology of Polish Academy of Sciences, 3 Pasteur, 02-093 Warszawa, Poland, anna.gieryng@gmail.com
MINOCYCLINE REDUCES PRODUCTION OF TUMOR-DERIVED OSTEOPONTIN/SPP1 AND MODULATES THE IMMUNE MICROENVIRONMENT OF RAT C6 GLIOMAS
Gieryng A.; Ellert-Miklaszewska A.; Pilanc P.; Ochocka N.; Kaza B.; Kaminska B.
Keywords: GAM reprogramming; Osteopontin Spp1; Minocycline Mino

34. Göttig, Tatjana
Department of Neurosurgery, Friedrich-Schiller-University Hospital, Jena, Bonhoefferstraße 2, 07747 Jena, Tatjana.goettig@gmail.com
MAPK- AND PI3K/MTOR- SIGNALING CASCADE: GENE EXPRESSION ANALYSIS IN HUMAN GLIOMA IN RELATION TO ITS CLINICAL RELEVANCE
Göttig, T; Freitag, D; Kalff, R; Walter, J.
Keywords: Human Glioma; MAPK- and PI3K/mTOR- Signaling cascade; Gene expression

35. Granberg, Kirsi
BioMeciTech, University of Tampere, Sipil kuja 5, 33820 Tampere, Finland, kirsi.granberg@gmail.com
STRONG FGFR3 STAINING IS A MARKER FOR FGFR3 FUSIONS IN DIFFUSE GLIOMAS
Granberg, K.J.; Annala, M.; Lehtinen, B.; Kesseli, J.; Haapasalo, J.; Ruusuvuori, P.; Yli-Harja, O.; Visakorpi, T.; Haapasalo. H, Nykter, M., Zhang, W.
Keywords: gene fusion; biomarker; glioblastoma

36. Grube, Susanne
Department of Neurosurgery, University Hospital Jena, Am Klinikum 1, 07747 Jena, susanne.grube@med.uni-jena.de
IS EGCG A USEFUL ANTIOXIDANT SUPPLEMENTATION TO FIGHT GLIOMAS?
Grube, S.; Kögler, Ch.; Freitag, D.; Walter, J.; Ewald, C.; Kalff, R.
Keywords: glioblastoma; EGCG; habituation

37. Gu, Song
Dept. Neurosurgery of First Affiliated hospital of Anhui Medical University, Jixi Road 218, 230022 Hefei, China
TRANSCRIPTION FACTOR PU.1 IS INVOLVED IN THE PROGRESSION OF GLIOMA
Song G, Meiqing L, Yuanzhi X
Keywords: PU.1; Glioma; P2XR7

38. Gupta, Bhavana
European Cancer Stem Cell Research Institute, Cardiff University, Maindy Road, CF24 4HQ Cardiff, UK, guptab@cardiff.ac.uk
LIVE CELL IMAGING OF TUMOR-ASTROCYTE INTERACTIONS
Gupta, B.; Hogan, C.; Siebzehnrubl, F.A.
Keywords: Astrocyte reactivity; Tumor-astrocyte interactions; Co-culture system

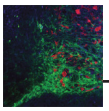
39. Haage, Verena Claudia
Cellular Neurosciences, Max-Delbrück-Cetrum für Molekulare Medizin, Robert-Rössle-Str. 10, 13125 Berlin, verena.haage@mdc-berlin.de
HDAC EXPRESSION PATTERN IN GLIOMA-ASSOCIATED MICROGLIA
Verena Haage; Andreas Faissner; Susanne Wolf; Helmut Kettenmann
Keywords: HDAC; Glioma-associated Microglia; Epigenetics

40. Haehnel, Susann
Anatomy, Oststrasse 25, 04317 Leipzig, , susann.haehnel@medizin.uni-leipzig.de
ORGANOTYPIC GLIOBLASTOMA TISSUE SLICES CAN BE ANALYZED BY RNA SEQUENCING, WHOLE SLICE HISTOLOGICAL ANALYSIS AND IMMUNOBLOTTING
Haehnel, S.; Reiche, K.; Winter, K.; Blumert, C.; Oppermann, H.; Meixensberger, J.; Bechmann, I.; Gaunitz, F.

41. Hahn, Mirja
Neurosurgery, University of Wü□rzburg, Josef-Schneider-Strasse 11, 97080 Würzburg, mirja.hahn@stud-mail.uni-wuerzburg.de
AUXILIARY EFFECT OF TUMOR-TREATING-FIELDS IN CONJUNCTION WITH MITOTIC CHECKPOINT INHIBITION ON GLIOBLASTOMA CELLS
M. Hahn; A.F. Kessler; F. Gross; G.E. Frömbling; R.-I. Ernestus; M. Löhr; C. Hagemann
Keywords: Glioblastoma; TTFields; Mitotic checkpoint

42. Hils, Nora
Johann-Joachim-Becher-Weg 15, 55128 Mainz, nhils01@uni-mainz.de
BEHAVIOR OF HUMAN GLIOBLASTOMA CELL LINES IN DIFFERENT HYPOXIC CONDTIONS
Hils, Nora; Oancea-Castillo, Liliana; Stradmann-Bellinghausen, Beate; Régnier-Vigouroux, Anne
Keywords: Hypoxia; 3D model; Lactate

43. Hou, Mengzhuo
Neurosurgical Research, Ludwig-Maximilians-University of Munich, Marchioninistr.15, 81377 Munich, jackson0086@163.com
INHIBITION OF APLN-APLNR SIGNALLING BLOCKS TUMOUR ANGIOGENESIS AND ATTENUATES INVASIVE GLIOBLASTOMA GROWTH
M.Hou, G.Mastrella, A.Jarczewski, V.Stöcklein, S.Kälin, M.Volmar, R.Monk, Y.Li, H.Miletic, C.Herold-Mende, A.Martin-Villalba, G.Gargiulo, M.Synowitz, A.L. Vescovi, J.M. Penninger, R.Bjerkvig, U.Schüller, J.-C.Tonn, F.Heppner, R.Glass1, .R.E. Kälin
Keywords: APLN-APLNR signalling; angiogenesis; GBM cell invasion



44. Hutter, Gregor
Neurosurgery, University Hospital Basel, Spitalstrasse 21, 4031 Basel, Switzerland, gregorhutter1@gmail.com
CD47-SIRPA BLOCKADE INDUCES A MICROGLIAL PHENOTYPIC SHIFT AND PROMOTES ACTIVE GLIOBLASTOMA HAGOCYTOSIS IN VIVO
G. Hutter, J. L. Theruvath, C.-M. Graef, M. Zhang, I. L. Weissman, S. S. Mitra, S. H. Cheshier
Keywords: glioblastoma; microglia; immunotherapy

45. Ismer, Britta
DKFZ, Im Neuenheimer Feld 580, 69120 Heidelberg, , b.ismer@dkfz.de
GENERATION, CHARACTERISATION AND TREATMENT OF NOVEL MURINE MODELS FOR HUMAN PEDIATRIC GLIOMA
Ismer, B.; Moshe, I.; Gronych, J.; Friedmann-Morvinski, D.; Jones, D. T. W.
Keywords: Mouse models; Pediatric Glioma;

46. Jacobs, Kathryn
CRCINA, University of Nantes, 8 Quai moncousou, 44000 Nantes, France, kathryn.jacobs@etu.univ-nantes.fr
NEUTRALIZING GP130 INTERFERES WITH ENDOTHELIAL-MEDIATED EFFECTS ON GLIOBLASTOMA STEM-LIKE CELLS
Jacobs, KA.; Hartford-Wright, E.; Gavard, J.
Keywords: GP130; GSCs; Gliblastoma Multiform

47. Jimenez Pascual, Ana
School of Bioscience, European Cancer Stem Cell Research Institute ECSCRI - Cardiff University, UK, JimenezPascualA@cardiff.ac.uk
MICROENVIRONMENTAL FGF2 INDUCES ZEB1 EXPRESSION IN GLIOBLASTOMA
Jimenez Pascual, A.; Holzmann, K.; Siebzehnrubl, F.A.
Keywords: Glioblastoma; FGF2; ZEB1

48. Johansson, Elinn
Translational Cancer Research TCR, Laboratory Medicine, Lund University, 223 63 Lund, Sweden, elinn.johansson@med.lu.se
CD44 INTERACTS WITH HIF-2ALPHA TO MODULATE THE HYPOXIC PHENOTYPE OF PERINECROTIC AND PERIVASCULAR GLIOMA CELLS
Johansson E.; Pantazopoulou V.; Grassi E; Lindgren D.; Axelson H; Pietras A.
Keywords: CD44; HIF-2alpha; stem cell niche

49. Kälin, Roland
Neurosurgical Research, LMU University Clinics, Marchioninistr. 15, 81377 München, roland.kaelin@med.uni-muenchen.de
NEWLY IDENTIFIED PERICYTE-PROGENITOR CELLS PROMOTE GBM-ANGIOGENESIS
Roland E. Kälin, Yuping Li, Yingxi Wu, Katharina Eisenhut, Eloi Montanez, Jörg-Christian Tonn, Michael Synowitz, Rainer Glass
Keywords: pericyte, progenitor, GBM angiogenesis

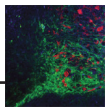
50. Kaffes, Ioannis
Charité-Universitätsmedizin Berlin, Plantagenstraße 17, 12169 Berlin, ioannis.kaffes@charite.de
MESENCHYMAL GLIOBLASTOMAS ARE CHARACTERIZED BY AN INCREASED IMMUNE CELL INFILTRATION COMPARED TO PRONEURAL AND CLASSICAL TUMORS
Kaffes I.; Szulzewsky F.; Chen Z.; Herting C.; Vel zquez Vega J.; Shelton J.; Switchenko J. et al.
Keywords: Glioblastoma; Subtype; Microenvironment

51. Kaube, Nelli
Department for Neurosurgery, Am Klinikum 1, 07747 Jena, newyork31@gmx.de
INFLUENCE OF LOW DOSE ORLISTAT ON GLIOBLASTOMAS‘ FATTY ACID METABOLISM
Kaube, N., Grube, S., Walter, J., Ewald, C., Kalff, R.
Keywords: Gliobastoma; Orlistat;

52. Krassnig, Stefanie
Department of Pathology, Medical University Graz, Auenbruggerplatz 25, 8036 Graz, Austria, stefanie.krassnig@medunigraz.at
EUKARYOTIC INITIATION FACTORS MIGHT REPRESENT A NOVEL MARKER TO MONITOR THE EFFECTIVENESS OF GLIOMA THERAPY
Stefanie Krassnig, Andrea Orthmann, Christina Wohlrab, Nicole Golob-Schwarzl, Anna Toeglhofer, Christina Wodlej, Mirjam Pennauer, Andrea Raicht, Kariem Mahdy Ali, Gord von Campe, Margit Gogg-Kamerer, Marlene Leoni, Stephan Sygulla, Jens Hoffmann, Serge Weis, Martin Benesch, Johannes Haybaeck
Keywords: Eukaryotic initiation factors; glioma; temozolomide

53. Krenzlin, Harald
Neurosurgery, Brigham and Women‘s Hospital, Harvard Medical School, 60 Fenwood Road, Boston, USA, h.krenzlin@t-online.de
CMV INFECTION STIMULATES TUMOR CELL – PERICYTE CROSSTALK TO FACILITATE ANGIOGENESIS AND MIGRATION IN GLIOBLASTOMA
Harald Krenzlin, Korneel Grauwet, Hong Zhang, Pranja Behera, Marion B Griessl, Michael Gutknecht, Charles H Cook, E Antonio Chiocca, Sean Lawler

54. Krona, Cecilia
Immunology, Genetics and Pathology, Uppsala University, 75185 Uppsala, Sweden, cecilia.krona@igp.uu.se
SYSTEMATIC XENOTRANSPLANTATION OF GLIOMA STEM CELL LINES FOR PHARMACOLOGICAL STUDIES ACROSS A WIDE RANGE OF TUMOR BEHAVIORS
Krona, C; Kundu, S; Holmberg-Olausson, K; Islam, R; Ramachandra, R; Elfineh, L; Nelander, S
Keywords: Glioma; Mouse model; glioma stem cells



55. Kundu, Soumi
Immunology Genetics and Pathology, Uppsala University, Dag Hammerskjälds väg 20, 75185 UPPSALA, Sweden, soumi.kundu@igp.uu.se
EFFECT ON GLIOMA CELL VIABILITY BY ENHANCED PROTEIN LOAD STRESS FOLLOWING PROTEASOME INHIBITION
Kundu, S.; Awe, O.; Schmidt, L.; Baskaran, S.; Johansson, P.;Elfineh, L.; Krona, C.; Nelander, S.
Keywords: proteasome inhibition; proteotoxic stress; cellular metabolism

56. Langella, Tiziana
Unit of Molecular Neuro-oncology, Neurological Institute Carlo Besta, Via Celoria 11, 20133 Milano, Italia, tiziana.langella@istituto-besta.it
RNA SEQUENCING OF GL261 CELLS AND TUMORS AND TCGA DATA POINT TO CD74 AS A MAJOR PLAYER IN TUMORIGENIC GLIOMA MICROENVIRONMENT
Tiziana Langella, Jiguang Wang, Serena Pellegatta, Antonio Iavarone, Raul Rabadan, Gaetano Finocchiaro
Keywords: GL261; sequencing; glioma microenvironment

57. Li, Yuping
Neurosurgical Research, University Clinics, Ludwig-Maximilians University Munich, Marchioninistr. 15, 81377 München, dr.yuping.li@gmail.com
DIFFERENT PROMOTER ELEMENTS OF THE NESTIN GENE PROVIDE LINEAGE TRACING MODELS FOR NEURAL PRECURSORS OR PERICYTE PROGENITORS
Li, Y.; Kälin, RE.; Glass R
Keywords: Nestin; neural precursors; pericyte progenitors

58. Linder, Benedikt
Neuroscience Center, University Hospital Frankfurt, H.-Hoffmann-Strasse 7, 60528 Frankfurt/M., linder@med.uni-frankfurt.de
TARGETING THE HEDGEHOG-SIGNALING PATHWAY IN GLIOMA STEM-LIKE CANCER CELLS
Linder, B.; Kögel, D.
Keywords: glioblastoma; Hedgehog; glioma stem-like cells

59. Lyons Rimmer, Jade
Peninsula School of Medicine and Dentistry, Plymouth University, 21 Lisson Grove, Plymouth, jade.lyonsrimmer@plymouth.ac.uk
THE POTENTIAL OF CRL4DCAF1 AND KSR1 AS THERAPEUTIC TARGETS IN MERLIN-DEFICIENT TUMOURS
Lyons Rimmer J, Baiz D, Ercolano E, Zhou L, Hilton D, Hanemann CO

60. Magod, Prerna
Biochemistry and Molecular Biology, Tel Aviv University, Klausner street, 6997801 Tel Aviv, Israel, prernamagod@gmail.com
UNVEILING THE CELLULAR AND MOLECULAR CHANGES OF THE MICROENVIRONMENT DURING BRAIN TUMOR DEVELOPMENT
Magod P.; Agemy, L; Rousso-Noori,L; Friedmann-Morvinski,D
Keywords: Glioblastoma; Tumor microenvironment; Dedifferentiation

61. Maire, Cécile
Neurosurgery, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20251 Hamburg, cmaire@uke.de
OPTICAL BARCODING: A NEW TECHNIQUE TO ANALYZE TUMOR HETEROGENEITY
Cecile L. Maire, Malte Mohme, Kristoffer Riecken, Antonio-Virgilio Failla, Marlena Helms, Katharina Kolbe, Boris Fehse, Manfred Westphal, Katrin Lamszus
Keywords: GBM; heterogeneity; barcoding

62. Malatesta, Paolo
DIMES, University of Genoa, Largo Rosanna Benzi 10, 16132 Genoa, Italy, paolo.malatesta@unige.it
IMMUNOESCAPE DURING GLIOMA PROGRESSION
Malatesta Paolo, Appolloni Irene, Marubbi Daniela, Alessandrini Francesco, Ceresa Davide
Keywords: PDGF-B; CD45; gene expression profile

63. Malric, Laure
Cancer Research Center of Toulouse CRCT, INSERM U1037, 2 avenue Hubert Curien - CS 53717, 31037 Toulouse Cedex 1, France, laure.malric@inserm.fr
ROLE OF A BETA INTEGRIN IN STEMNESS MAINTENANCE AND RADIORESISTANCE OF GLIOBLASTOMA-INITIATING CELLS
Malric, L.; Monferran, S.; Dahan, P.; Delmas, C.; Lubrano, V.; Kowalski-Chauvel, A.; Toulas, C.; Cohen-Jonathan Moyal, E., Lemarié, A.
Keywords: Beta integrin; Glioblastoma; Cancer stem cells

64. Mark, Georg
Neurosurgery, Carl Gustav Carus University Hospital, TU Dresden, Dr.-Otto-Nuschke Str. 8, 01809 Heidenau, Georg.Mark@uniklinikum-dresden.de
LABEL-FREE MULTIPHOTON IMAGING FOR RECOGNITION OF HUMAN BRAIN TUMOR BORDERS BY TEXTURE ANALYSIS
Mark, G.; Uckermann, O.; Galli, R.; Meinhardt, M.; Steiner, G.; Schackert,G.; Kirsch, M.
Keywords: tumor borders; multiphoton imaging; label-free

65. Martellotta, Donato Daniel
Department of Neurosurgery, University Hospital Würzburg, Josef-Schneider-Strasse 11, 97080 Würzburg, ddmartellotta@libero.it
CXCR4- A NEW PLAYER IN VESTIBULAR SCHWANNOMA PATHOGENESIS
D. Martellotta, M. Hummel, A. Schwerdtfeger, A. Kessler, J. Perez, C. Monoranu, R.-I. Ernestus, C. Matthies, M. Löhr, C. Hagemann
Keywords: vestibular schwannoma; CXCR4

66. Mastrella, Giorgia
Clinic for Neurosurgery, University Clinics, Ludwig Maximilians University, Marchioninistraße 15, 81377 Munich, Giorgia.Mastrella@med.uni-muenchen.de
PRIMARY GLIOBLASTOMA CELLS EXPLOIT APLN-APLNR SIGNALLING TO ATTRACT TUMOUR ASSOCIATED MYELOID CELLS
Mastrella, G.; Glass, R.; Kälin, R.E.
Keywords: APLN-APLNR signalling; tumour-associated myeloid cells; glioblastoma

67. McAbee, Joseph
Clinical Neurosciences, University of Cambridge, St. John's College, CB2 1TP Cambridge, UK, mcabeejh@gmail.com
OVERCOMING THE BLOOD BRAIN BARRIER AND GENOMIC COMPLEXITY
McAbee, J.H.; Parkins, C.C.; Scherman, O.A.; Fisher, J.; Watts, C.
Keywords: Local drug delivery for GBM; Blood brain barrier; Computational modeling

68. Mecca, Carmen
Experimental Medicine, University of Perugia, Piazza Lucio Severi n.1, 6132 Perugia, Italy, carmecca@gmail.com
MTOR PATHWAY IN GLIOBLASTOMA MULTIFORME: AN IN VITRO STUDY OF PP242, A NOVEL MTOR INHIBITOR
Mecca, C; Ragonese, F; Fioretti, B; Cataldi, S; Albi, E; Donato, R; Arcuri, C.
Keywords: mTOR; PP242; Glioblastoma multiforme

69. Michen, Susanne
Klinik und Poliklinik für Neurochirurgie, Universitätssklinikum Carl Gustav Carus Dresden, Fetscherstraße 74, 01307 Dresden, susanne.michen@uniklinikum-dresden.de
AN ANTIBODY-GUIDED POLY-PROPYLENE-IMINE PPI-BASED POLYPLEX-SYSTEM FOR SIRNA-TREATMENT OF EGFRVIII-POSITIVE TUMORS
Michen, S.; Tietze, S.; Ennen, F., Janke, A., Schackert, G.; Appelhans, D.; Temme, A.
Keywords: siRNA-treatment; polyplex; EGFRvIII

70. Miletic, Hrvoje
K. G. Jebsen Brain Tumour Research Centre, Department of Biomedicine, University of Bergen, Norway, hrvoje.miletic@uib.no
LONG-TERM PRODRUG ADMINISTRATION IMPROVES LENTIVIRAL VECTOR MEDIATED SUICIDE GENE THERAPY
Jubayer A Hossain, Lars Rømo Ystaas, Krishna M Talasila, Sandra Ninzima, Kristoffer Riecken, Boris Fehse, Rolf Bjerkvig and Hrvoje Miletic
Keywords: glioblastoma, suicide gene therary, EGFR

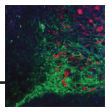
71. Moghaddaskho, Farima
Hematology/Oncology & Stem Cell Transplantation Research Center, Shariati Hospital, Jalal-e-Ale-Ahmad, 14111 Tehran, Iran, farima_moghadaskho@yahoo.com
EFFECTS OF TIVOZANIB, A PAN-INHIBITOR OF VEGF RECEPTORS, ON GROWTH AND INVASIVE ABILITIES OF GLIOMA CELLS
Farima Moghaddaskho, Majid Momeny, Narges K. Gortany, Hassan Yousefi, Zahra Sabourinejad, Ghazaleh Zarrinrad, Haniyeh Eyvani, Farinaz Barghi, Azam Zaghal, Ghazaleh Sankanian, Fatemeh Esmaeili3, Zivar Alishahi, Hoda T. Moghaddam, Somayeh Vaezi-Joze, Mahmoud Ghazi-Khansari, Kamran Alimoghaddam, Ardeshir Ghavamzadeh, Seyed H. Ghaffari
Keywords: Glioblastoma; VEGF family; Tivozanib

72. Moriconi, Chiara
School of Pharmacy and Pharmaceutical Sciences, Cardiff University, King Edward VII Avenue, CF10 3NB Cardiff, UK, MoriconiC@cardiff.ac.uk
CAVEOLIN-1, A DRIVER OF INVASIVE PHENOTYPE IN IN-VITRO 3D-SPHEROID ASSAYS COMPRISED OF HIGH GRADE GBM CELLS ASSOCIATION WITH AN AKT-INHIBITED PHENOTYPE
Moriconi, C.; Palmieri, V; Tornillo, G.; Pilkington, G.;Gumbleton, M.
Keywords: Adult gliomas; Imaging; Invasion

73. Neirinckx, Virginie
Luxembourg Institute of Health, Val Fleuri 84, 1526 Luxembourg, Luxembourg, virginie.neirinckx@lih.lu
DECIPHERING LRIG1 TUMOR-SUPPRESSING SIGNALING IN GLIOMA
Neirinckx, V.; Schuster, A.; Hedman, H.; Niclou, S.P.
Keywords: Glioblastoma; Leucine rich repeat and immunoglobulin-like domain; Receptor tyrosine kinase

74. Nelander, Sven
Dept of Immunology, Genetics and Pathology, Rudbeck Lab, Uppsala University, Dag Hammarskjölds v 20, 75185 Uppsala, Sweden, sven.nelander@igp.uu.se
THE HGCC FUNCTIONAL ATLAS UNCOVERS MODULAR NETWORKS OF DRUG SENSITIVITY IN GLIOBLASTOMA CELLS
Johansson, P.; Schmidt, L.; Baskaran, S.; Kundu, S.; Awe, O. Matuzewski, D.; Elfineh, L.; Häggblad, M.;Mertens, U.; Kastemar, M.
Keywords: global model of drug response in glioblastoma; patient-derived glioblastoma cancer stem cells; systems biology

75. Neumann, Julia E.
Institute for Neuropathology, University Hospital Hamburg, Veilchenweg 18, 22529 Hamburg, Julia.E.Neumann@gmx.de
SIMULTANEOUS ACTIVATION OF SHH- AND WNT-SIGNALING IN NEURAL PROGENITORS DRIVES FORMATION OF EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES ETMR AND INDICATES POTENTIAL THERAPEUTIC AVENUES
Julia E. Neumann, Annika K. Wefers, Sander Lambo, Edoardo Bianchi, Marie Bockstaller, Mario M. Dorostkar, Valerie Meister, Pia Schindler , Andrey Korshunov, Katja von Hoff , Johannes Nowak, Monika Warmuth-Metz, Marlon R. Schneider, Ingrid Müller-Renner, Daniel J. Merk, Mehdi Shakarami, Rainer Glass, Jennifer A. Chan, M. Mark Taketo, Philipp Neumann, Marcel Kool and Ulrich Schüller
Keywords: ETMR; Sonic hedgehog SHH; Mouse model



76. Noelanders, Rivka
Unit of developmental biology, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
MODELLING MOLECULAR SUBTYPES OF MEDULLOBLASTOMA IN XENOPUS TROPICALIS USING CRISPR/CAS9
Rivka Noelanders, Dionysia Dimitrakopoulou, Thomas Naert, Tom Van Nieuwenhuysen, Imane El Fakhar and Kris Vleminckx
Keywords: Medulloblastoma; CRISPR/Cas9; Xenopus

77. Oancea-Castillo, Liliana
Institute of Developmental Biology & Neurobiology, Johannes Gutenberg Universität-Mainz, Johann-Joachim-Becher-Weg 13-15 / 1.OG R.01-5, 55128 Mainz, l.cisneros@uni-mainz.de
IL-6: KEY MODULATOR IN GLIOMA IMMUNE MICROENVIRONMENT
Oancea-Castillo, L; Régnier-Vigouroux, A.
Keywords: IL-6; STAT-3; Three-dimensional model

78. Orthmann, Andrea
EPO GmbH Berlin, Robert-Rössle-Str. 10, 13125 Berlin, andrea.orthmann@epo-berlin.com
NEW PRECLINICAL MODELS FOR NEURO-ONCOLOGY: INTEGRATING DATA FROM PD3D® MODELS AND ORTHOTOPIC XENOGRAFTS
A.Orthmann; J. Haybaeck; A. Jödicke; M. Linnebacher; A. Silvestri; C. Regenbrecht; I. Fichtner; J. Hoffmann
Keywords: PDX; PD3D; GBM modeling

79. Pagenstecher, Axel
Neuropathologie, Universität Marburg, Baldingerstrasse, 35043 Marburg, , pagenste@med.uni-marburg.de
CONDITIONALLY PROLIFERATING PRIMARY MURINE ASTROCYTES RECAPITULATE CHARACTERISTICS OF HUMAN GLIOBLASTOMA
Krüger KNP, Bortolussi G, Weissbarth G, Pagenstecher A
Keywords: Glioma; mouse model;

80. Palmieri, Valentina
Physics Institute, Catholic University of Sacred Hearth, Largo Francesco Vito 1, 168 Roma, Italy, valentina.palmieri@unicatt.it
INSIDIA: IMAGEJ MACRO FOR HIGH-THROUGHPUT AND HIGH-CONTENT SPHEROID INVASION ANALYSIS
Palmieri, V.; Moriconi, C.; Di Santo, R.; Tornillo, G.; Papi, M.; Pilkington, G.; De Spirito, M.; Gumbleton, M.
Keywords: spheroids; quantitativa analysis; ImageJ Macro

81. Park, Jongwhi
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SPROUTY2 ENHANCES TUMOR-PROPAGATING POTENTIAL OF GLIOMA CELLS
Jongwhi Park1, Guido Wollmann2, Barbara Fogli1, Felipe Trivik-Barrientos1, Carles Rodriguez Urbiola2, Stephan Geley3, Lars Klimaschewski1

82. Pethö, Zoltán
Department of Biophysics and Cell Biology, University of Debrecen, Poroszlay str 112., 4032 Debrecen, Hungary, petho.zoltan@med.unideb.hu
KCA1.1 CHANNEL AUXILIARY BETA SUBUNIT COMPOSITION IN GLIOBLASTOMA MULTIFORME
Zolt n Peth?; µlmos Klekner; L szlo Bogn r; Zolt n Varga; György Panyi
Keywords: glioblastoma; ion channel; auxiliary subunits

83. Pires Afonso, Yolanda
Department of Oncology, Luxembourg Institute of Health, 84, Val Fleuri, L-1526 Luxembourg, Luxembourg, yolanda.piresafonso@lih.lu
CHARACTERIZATION OF MICROGLIA/MACROPHAGE PHENOTYPES IN GLIOBLASTOMA PATIENT-DERIVED XENOGRAFT MOUSE MODELS
Pires-Afonso, Y; Golebiewska, A; Oudin, A; Michelucci, A; Niclou, SP
Keywords: Tumor-associated microglia/macrophages; Patient-derived xenograft; Glioblastoma

84. Purcz, Katharina
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IMIDAZOLE CONTAINING COMPOUNDS ARE POTENTIAL DRUGS FOR THE TREATMENT OF GLIOBLASTOMA
Purcz, K.; Seidel, C.; Birkemeyer, C.; Dietterle, J.; Meixensberger, J.; Gaunitz, F.; Oppermann, H.
Keywords: glioblastoma; carnosine; L-histidine

85. Rezk, Rasha
Engineering, University of Cambridge, Trumpington street, Cb2 1PZ Cambridge, UK, rsr33@cam.ac.uk
MORPHOLOGICAL AND ADHESION DIVERSITY OF PRIMARY GBM
R. Rezk, A. Wendler , P. Humphreys, C. Watts, A.E. Markaki
Keywords: Glioblastoma; Adhesion strength; Cell migration

86. Sander, Caroline
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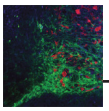
GENETIC ANALYSES ON GLIOBLASTOMA STEM LIKE CELLS AND GLIOBLASTOMA TISSUE USING SNP ARRAY AND GENE EXPRESSION
Sander, C; Wallenborn, M.; Kirsten, H.; Xu, L.-X.; Ahnert, P.;Krupp, W.; Meixensberger, J.; Holland, H.
Keywords: GSCs; Genetic analyses



87. Schuster, Anne
Oncology, Luxembourg Institute of Health, 84, Val Fleuri, 1526 Luxembourg, Luxembourg, anne.schuster@lih.lu
CHARACTERIZATION OF GLIOBLASTOMA CELL INVASION: TOWARDS NOVEL THERAPEUTIC TARGETS
Schuster, A.
Keywords: glioblastoma; invasion; patient-derived GBM cell lines

88. Semtner, Marcus
Cellular Neurosciences, MDC Berlin, Robert-Roessle-Str. 10, 13092 Berlin, marcus.semtner@mdc-berlin.de
SPONTANEOUS CA2 TRANSIENTS IN MOUSE MICROGLIA
Korvers L; de Andrade Costa A; Mersch M; Matyash V; Kettenmann H; Semtner M.
Keywords: microglia; calcium; spontaneous

89. Sharma, Ira
Tumor Biology, National Institute of Pathology, Lab no. 611, 6th floor, Safdarjung Hospital C, 110029 Delhi, India, irasharma99@gmail.com
ROLE OF CXCL8-CXCR1/2 AXIS IN GLIOBLASTOMA CELL PROLIFERATION, INVASION AND VASCULAR MIMICRY
Sharma I.; Singh A.; Saxena S.
Keywords: CXCL8; CXCR1; CXCR2

90. Siebzehnrubl, Florian
European Cancer Stem Cell Research Institute, Cardiff University, Maindy Road, CF24 4HQ Cardiff, UK, fas@cardiff.ac.uk
SLOW DIVIDING GLIOBLASTOMA STEM CELLS DEPEND ON LIPID METABOLISM AND MITOCHONDRIAL FUNCTION
Siebzehnrubl, F.A.; Hoang-Minh, L.B.; Schmoll, M.; Amin, K.; Dajac, K.; Vuong, A.; Huang, J.; Yang, C.; Garrett, T.; Sarkisian,M. R., Reynolds, B.A., Deleyrolle, L. P.
Keywords: cancer stem cells; metabolism; therapy resistance

91. Simon, Michèle
Department of Neurosurgery, Vivantes-Klinikum im Friedrichshain, Landsberger Allee 49, 10249 Berlin, michele.simon@vivantes.de
THE MIR-143/145 CLUSTER AS CRITICAL REGULATOR IN GLIOMAGENESIS?
Simon, M.; Freitag, D.; Moskopp, D.; Kalff, R.; Walter, J.
Keywords: Glioma; mTOR; miRNA

92. Soubéran, Aurélie
Inserm UMR_911, Center for Research in Oncobiology and Oncopharmacology, 27 Bd Jean Moulin, 13005 Marseille, FRANCE, aureliesouberan@gmail.com
DIFFERENTIAL EFFECTS OF SMAC MIMETIC GDC-0152 IN GLIOBLASTOMAS DEPENDING ON TUMOR MICROENVIRONMENT
Soubéran, A; Cappa, J; Raujol, J; Chocry, M; Baeza-Kallee, N; Colin, C; Figarella-Branger, D; Tchoghandjian, A.
Keywords: Glioblastomas; Stem cells; Smac mimetic

93. Spyrou, Argyris
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TARGETING HEPARAN SULFATE IN THE BRAIN TUMOR MICROENVIRONMENT
Spyrou, A.; Kundu, S.; Wicher, G.; Xiong, A.; Haseeb, L.; Ilan, N.; Vlodavsky, I.; Li, J-P; Forsberg-Nilsson, K.
Keywords: heparan sulfate; heparanase; brain tumor

94. Steinbach, Tanja
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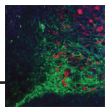
COMPARING EXPRESSION ANALYSIS OF THE KEY PROTEINS OF THE PI3K/MTOR-SIGNALING PATHWAY IN HUMAN MENINGIOMA AND GLIOMA
T.Steinbach, D. Freitag, R. Kalff, J. Walter
Keywords: meningeoma; glioma; PI3K/mTOR signaling

95. Steinmann, Anna
Functional Neuroanatomy, Institute of Anatomy and Cell Biology, Im Neuenheimer Feld 307, 69120 Heidelberg, steinmann@ana.uni-heidelberg.de
ROLE OF GDF-15 IN HIGH GRADE GLIOMA
Steinmann, A.; Eisemann, T.; Kuner, T.; Peterziel, H.; Strelau, J.
Keywords: TGF-á; GDF-15; Tumor microenvironment

96. Stepniak, Karolina
Neurobiology Center, Nencki Institute of Experimental Biology, Pasteur 3, 02-093 Warszawa, Poland, k.stepniak@nencki.gov.pl
OPEN CHROMATIN AND EPIGENETIC LANDSCAPE OF HUMAN BENIGN AND MALIGNANT GLIOMAS
Stepniak K.; Macioszek A.; Wojtas B.; Gielniewski B.; Czernicki T.; Czapski B.; Grajkowska W.; B. Wilczynski; Kaminska B.
Keywords: glioblastoma; epigenetics; geneome-wide studies

97. Stradmann-Bellinghausen, Beate
Institute for Developmental Biology and Neurobiology, Johannes Gutenberg University-Mainz, Johann-Joachim-Becher-Weg 15, 55128 Mainz, bstradma@uni-mainz.de
ANALYSIS OF IDH1 AND L-PLASTIN IN HUMAN MICROGLIA/MACROPHAGES EXPOSED TO GLIOBLASTOMA CELLS
Stradmann-Bellinghausen, B.; Choi, J.; Ruppert, T.; Neumann, H.; Régnier-Vigouroux, A.
Keywords: proteomics; tumor-associated microglia/macrophages; metabolism



98. Tantillo, Elena
Neuroscience, Scuola Normale Superiore, via salvo d'acquisto 6, 56031 Bientina, Italy, elenatantillo@gmail.com
BIDIRECTIONAL NEURON-GLIOMA INTERACTIONS: EFFECTS OF GLIOMA CELLS ON SYNAPTIC ACTIVITY AND ITS IMPACT ON TUMOR GROWTH
Tantillo, E;Cerri, C;Olimpico, F;Mazzanti, CM;Caleo, M.
Keywords: Glioma; Tumor proliferation; Neural activity

99. Trivik-Barrientos, Felipe
Neuroanatomy, Neurosurgery, Medical University of Innsbruck, Christoph-Probst-Platz 1, Innrain 52, 6020 Innsbruck, Austria, felipe.trivik@student.i-med.ac.at
SPROUTY2: AN IMPORTANT MODULATOR OF SIGNALING AND FGF RECEPTOR TRAFFICKING IN GLIOBLASTOMA MULTIFORME
Trivik-Barrientos, F.; Irschick R.; Ecker J.; Hausott B.; Klimaschewski L.
Keywords: Sprouty2; Glioblastoma; FGFR

100. Uckermann, Ortrud
Klinik und Poliklinik für Neurochirurgie, Universitätsklinikum Dresden, Fetscherstr. 74, 01307 Dresden, , ortrud.uckermann@uniklinikum-dresden.de
RAMAN SPECTROSCOPY FOR INTRAOPERATIVE DIAGNOSIS OF BRAIN TUMORS
Uckermann, O.; Galli, R.; Meinhardt, M.; Steiner, G.; Koch, E.; Schackert, G.; Kirsch, M.
Keywords: Raman spectroscopy; intraoperative; biopsy

101. Unger, Frank
Neurosurgery, Med Uni Graz, Auenbruggerplatz, 8036 Graz, Austria, frank.unger@klinikum-graz.at
COMBINED ENDOSCOPIC AND RADIOSURGICAL TREATMENT OF ESTHESIOBEUROBLASTOMA
F. Unger, C. Walch, H. Stammberger
Keywords: Endoscopic sinus surgery; olfactory neuroblastoma esthesioneuroblastoma; stereotactic radiosurgery

102. Uyar, Ramazan
Neurosurgical Research, Klinikum Grosshadern - LMU, Munich, Marchioninistr. 15, 81377 München, Ramazan.Uyar@med.uni-muenchen.de
GLIOMA-ASSOCIATED MESENCHYMAL STEM CELLS PROMOTE TUMOUR CELL INVASION
Uyar, R.; Volmar, MNM; Mastrella, G.; Kaelin, RE,;Glass, R.
Keywords: Mesenchymal Stem Cells; Invasion; EFNA3

103. Vannini, Eleonora
Hunimed University and Institute of Neuroscience CNR, Via dei Mortuli, 16, 50142 Firenze, Italy, eleo.vannini@gmail.com
THE DUAL EFFECT OF CNF1 ON GLIOMA: ANTI-NEOPLASTIC AGENT AND GUARDIAN OF BRAIN FUNCTION
Vannini, E.; Olimpico, F.; Maltese, F.; Costa, M.; Baroncelli, L.; Caleo, M.
Keywords: glioma; mouse model; CNF1

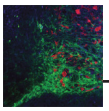
104. Volmar, Marie Nhery Murielle
Neurosurgical research, Klinikium Grosshadern, LMU, Marchioninistrasse 15, 81377 Munich, murielle.volmar@hotmail.fr
UNCOVERING PREDICTIVE GENETIC- AND METABOLIC-MARKERS FOR GBM THERAPY
Volmar M. N. M., Kälin R. E., Glass R.
Keywords: prediction; cannabidiol; GBM therapy

105. Willems, Estelle
GIGA-Neuroscience, University of Liége, Avenue Hippocrate, 15, 4000 Liége, Belgium, estelle.willems@doct.ulg.ac.be
ROLE OF AURORA KINASE A IN THE THERAPEUTIC RESISTANCE OF GLIOBLASTOMA ASSOCIATED WITH THE PRODUCTION OF CXCL12 IN THE SUBVENTRIC
Willems, E; Dedobbeleer, M; Digregorio, M; Lombard, A1,; Lumapat, P; Lambert, J; Szpakowska, M; Chevigné, A; Scholtes, F; Rogister, B
Keywords: Aurora A; CXCL12; Subventricular zones

106. Wu, Wei
Neuropathology, Technische Universität München, Ismaninger Str. 22, 81675 München, wei.wu@tum.de
THE CANCER STEM CELL FACTOR ALDEHYDE DEHYDROGENASE 1A3 ALDH1A3 IS REGULATED BY AUTOPHAGY IN HUMAN GLIOBLASTOMA CELLS
Wei Wu, Johannes Schecker, Jürgen Schlegel
Keywords: Glioblastoma; Cancer Stem Cells; Autophagy

107. Zhu, Yuan
Hufelandstr. 55, 45147 Essen, yuan.zhu@uk-essen.de
PROGRAMMED CELL DEATH 10 IN GLIOBLASTOMA
Zhu, Y
Keywords: PDCD10; angiogenesis; glioblastoma

108. Zollfrank, Julia
Department of Neurosurgery Charité Berlin, Simmelstr. 12, 13409 Berlin, , julia.zollfrank@charite.de
INHIBITION OF CXCR2/CXCL2 SIGNALING PATHWAY IN GLIOBLASTOMA MULTIFORME AS A THERAPEUTIC APPROACH
Zollfrank, J.; Acker, G.; Brandenburg, S.; Vajkoczy, P.
Keywords: glioblastoma; CXCR2-Antagonist; microglia



Abstracts of Poster Presentations

(in alphabetical order of presenting author as in list of poster presentations)

"REVERSE SIGNALING" OF THE TRANSMEMBRANE CHEMOKINE CXCL16 IN GLIOBLASTOMAS

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 # shared senior-authorship

Chemokines and their receptors play a decisive role in tumor progression and metastasis. We recently found a new signaling mechanism in glioblastomas (GBM) mediated by transmembrane (tm) chemokines that we termed "inverse signaling". Here, soluble (s)CXCL16 binds to the surface-expressed (tm)CXCL16, and induces signaling and biological effects in the stimulated cells, so that the transmembrane ligand itself acts as a receptor for its soluble counterpart. Now we hypothesized that (tm)CXCL16 is also capable of "reverse signaling", which means that the classical receptor CXCR6 binds to (tm)CXCL16 resulting in bidirectional responses in the receptor and in the ligand-exposing cells. As CXCL16 is frequently expressed, and CXCR6 can be found on distinct cell subpopulations in GBM in situ, this mechanism in question might take place in GBM. Initially, expression analysis yielded that CXCL16 was abundantly expressed in cultured human GBM cells, whereas CXCR6 was missing. After stimulation of (tm)CXCL16 positive GBM cells with recombinant (rec)CXCR6 protein, p42/44 kinase phosphorylation occurred and could be specifically abolished by CXCL16 RNAi knock down. Using (tm)CXCL16 and CXCR6 negative LOX tumor cells as a model system, specific p42/44 activation, NFκB translocation and biological responses resulted only in stably transfected CXCL16-positive LOX cells which were stimulated either with (rec)CXCR6 or generated CXCR6-positive LOX cell membranes. Effects were abolished when LOX clones with intracellularly truncated (tm)CXCL16 were used for described experiments. Thus, "reverse signaling" of transmembrane ligands may complement autocrine secondary signaling loops of classical receptors.

DORMANT HUMAN GLIOBLASTOMA CELLS OWN STEM CELL CHARACTERISTICS AND ARE DISTINCTLY AFFECTED BY CHEMOTHERAPEUTIC TREATMENT STRATEGIES

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Tumor dormancy is a phenomenon of malignant tumors which addresses a protected state which occurs at different stages of tumor progression. Striking parallels exist between the concept of tumor dormancy and the cancer stem cell theory. Both predict that a subset of tumor cells is responsible for tumor initiation, bears the ability to survive therapy, and may persist for longer time periods to cause delayed cancer recurrence and progression. We were able to show that proven dormancy markers [insulin-like growth factor-binding protein 5 (IGFBP5), ephrin receptor A5 (EphA5) and histone cluster 1 H2B family member k (H2BK)] were expressed in human glioblastomas (GBMs) in situ, were located in single tumor cells, could be co-stained witheach other and with the stem cell markers krüppel-like factor 4 (KLF4), octamer binding transcription factor 4 (OCT4) and sex determining region Y-box 2 (SOX2). Primary and commercial human GBM cultures were characterized by expression of individual, cell-type specific dormancy and stem cell markers, which were (up)regulated and could be co-stained in a cell-type specific manner upon long-term temozolomide (TMZ) treatment in vitro. At this, most GBM cells evolved a flat, large cell morphology. Depending on the individual cell-specific response to TMZ treatment, the signal kinases p38 and p42/44 were differentially activated, and a cell-type specific pattern of TMZ-induced and also combined TMZ/AT101-induced cytotoxicity was measured in different GBM cultures in vitro. Overall, we postulate that a better understanding of the dormant state of tumor cells is essential to further improve efficiency of treatment.

COMMON FEATURES BETWEEN EGFRVIII AND PDGF-B INDUCED GLIOMAS MODELS.

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1 DIMES - Università degli studi di Genova

2 IRCCS AOU S. Martino-IST Genova

Gliomas are among the hardest challenges in oncology. Alteration of EGFR or PDGF-B signalling is commonly observed in human glioblastomas. We investigated and compared the molecular profiles of two different mouse models of glioblastoma, induced by the alteration of these pathways. We overexpressed EGFRvIII receptor in embryonic neural progenitors of INK4a/Arf -/- mice, inducing the development of gliomas following transplantation in adult syngeneic animals. Cells from these tumors can be grown in vitro and serially re-transplanted in vivo giving rise to secondary tumors. We compared immunohistochemical, histopathological and genome-wide expression features of these tumors with a second glioma model induced by the overexpression of PDGF-B. Interestingly, the features of the two models are highly similar, although the penetrance of EGFRvIII-driven tumors is lower. Both models clearly share markers of the oligodendroglial lineage. The similarity is even more striking when considering the data obtained from microarray and NGS analyses. The comparison between our gene expression profiles with published datasets from neural cell types shows a strong similarity between oligodendrocyte progenitors (OPC) and EGFRvIII tumor cells which maps close to PDGF-B induced tumors but further from NSC. While the similarity between PDGF-B induced gliomas and OPCs fits with the role of PDGF-B in oligodendrocyte lineage specification, EFGR is less clearly connected with such cell lineages thus our results suggest a deeper and more general connection between OPC-like phenotype and gliomas.

TENASCIN-C AS A THERAPEUTIC TARGET FOR HIGH GRADE GLIOMAS

Anna-Maria Barciszewska1,2, Dariusz Wawrzyniak3, Monika Piwecka3, Malgorzata Szpakowska4, Stanislaw Nowak1,2, Katarzyna Rolle3

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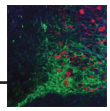
High grade gliomas (HGG) are aggressive brain tumors with frequent relapses and a high mortality, still remaining a diagnostic and therapeutic challenge. They present a highly invasive growth, rampant



genetic instability and intense resistance to apoptosis. HGG express a number of specific protein and RNA markers, that may be exploited as potential therapeutic targets in design of new treatment modalities based on nucleic acids. RNA interference (RNAi) is the most promising technique in the long line of nucleic acid-based therapeutic technologies. It has been shown that tenascin-C (TN-C), extracellular matrix protein, is overexpressed in glioblastoma and can be a good target in RNAi approach for its treatment. Using a long dsRNA complementary to TN-C (ATN-RNA), we conducted the experimental therapy of HGG patients. They show an improvement in quality of life and longer survival after ATN-RNA treatment compared to standard therapy. Among those receiving the dsRNA treatment, most had functional improvement scores. Our study shows that TN-C is a good therapeutic target in RNAi approach with a great impact on overall survival, progression free survival an quality of patients' life. Thus, targeting TN-C may be potentially helpful in designing more effective anti-cancer therapies, also used in conjunction with anti-proliferative modalities, such as chemotherapy, radiation therapy, and anti-angiogenesis strategies.

SYNTHETIC FLAVONOID COMPOUNDS INHIBIT GLIOBLASTOMA CELL PROLIFERATION AND VIABILITY

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1) Department of Biophysics and Cell Biology, University of Debrecen, Hungary, 2) MTA-DE NAP Ion ChannelStructure-Function Research Group, RCMM, Debrecen, Hungary

Although flavonoid compounds occur in plants and fungi as secondary metabolites, they also show biological activity in humans. It has already been described earlier that natural flavonoids (such as luteolin, orgenistein) selectively inhibit the growth of various tumor cells. Glioblastoma multiforme is the most common primary malignant brain tumor, in many cases showing poor prognosis. Our study aims to assess whether artificially modified flavonoids can inhibit the viability and proliferation in glioblastoma cells. In our experiments we used synthetically modified flavonoids produced by the Department of Organic Chemistry of the University of Debrecen. The chemical modifications mainly targeted the „A" ring on the flavones backbone on the 6' or 7' carbon atoms. We tested the compounds on the immortalized glioblastoma cell line U-87 MG. As control cells we used peripheral blood mononuclear cells. We measured the effect of more than 25 flavonoids on cellular viability after 3 days of treatment using propidium iodide staining on a FACS Array flow cytometer. We performed additional measurements with the most promising compounds where we tested the flavonoid induced dose and time dependent inhibition of cell proliferation by other methods such as the MTT assay. Altogether 15 of the available flavonoids caused pronounced cytotoxic effects. The compound coded PD-200 was the most potent flavonoid, that selectively and dose dependently showed a pronounced inhibition of tumor cell proliferation and viability on the U-87 MG cell line even at low micromolar concentrations. Our results suggest that the synthetic flavonoids showing biological activity may be included in chemotherapy in the future as supportive therapy.

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LAUROYL-GEMCITABINE LIPID NANOCAPSULES AS MULTI-DRUG COMBINATION NANOMEDICINE HYDROGEL FOR THE LOCAL TREATMENT OF GLIOBLASTOMA

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The local delivery of anti-cancer drug loaded hydrogels in the tumor resection cavity of Glioblastomas (GBM) is a promising strategy for the treatment of these incurable brain tumors. Indeed, reaching high local concentrations of the drug in the gap period between surgery and standard of care chemo-radiation could lead to a reduction of the formation of recurrences at the resection cavity borders. This research focuses on the use of Lauroyl-gemcitabine lipid nanocapsule (GemC12-LNC) as multi-drug combination nanomedicine hydrogel for this scope. The GemC12-LNC hydrogel was prepared by a phase-inversion technique process and characterized showing to be injectable, adapted for brain implantation and able to sustainably release the drug in vitro. In vivo, its tolerability has been demonstrated in healthy mice brain by TUNEL assay and Iba-1 immunostaining. The gel efficacy has been tested in mice using a U87 orthotopic GBM model and its resection model, showing increased median survival compared to the controls. Successively, the formulation has been optimized for the delivery of a second chemotherapeutic agent, Paclitaxel (PTX). The physicochemical and injectability properties of the new hydrogel did not significantly differ from the original one. In vivo, a 9L resection model of the GBM tumor has been developed in rats and efficacy studies are on-going after administration of PTX-GemC12-LNC gel in the resection cavity. In conclusion, our study presents a promising and innovative nanomedicine hydrogel loaded with two chemotherapeutic drugs for the local treatment of GBM. This gel has a very simple formulation, combines the properties of nanomedicines and hydrogels, and can be directly injected in the GBM resection cavity.

DIFFERENTIAL PROPAGATION OF STROMA AND CANCER STEM CELLS DICTATES TUMORIGENESIS AND MULTIPOTENCY

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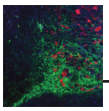
Glioblastoma Multiforme (GBM) is characterized by high cancer cell heterogeneity and the presence of a complex tumour microenvironment. Those factors are a key obstacle for the treatment of this tumor type. In this work, we performed high-throughput protein expression analysis and investigated the tumorigenicity of GBM-cells enriched under different culture conditions. We identified a marker panel that distinguished tumorigenic sphere cultures from non-tumorigenic serum cultures (high CD56, SOX2, SOX9, and low CD105, CD248, αSMA). Contrary to previous work, we found that „mixed cell cultures" grown in serum conditions are tumorigenic and express cancer stem cell (CSC) markers. As well, 1% serum plus bFGF and TGF-α preserved the tumorigenicity of sphere cultures and induced epithelial-to-mesenchymal transition (EMT) gene expression. Furthermore, we identified 12 genes that could replace the 840 genes of TCGA used for GBM-subtyping. Our data suggest that the tumorigenicity of GBM cultures depend on cell culture strategies that retain CSCs in culture rather than the presence of serum in the



cell culture medium and the 12-gene mini signature can be used for subtyping of GBM patients.

THE ROLE OF LET-7 MICRORNAS AS ACTIVATORS OF GLIOMA-ASSOCIATED MICROGLIA/MACROPHAGES

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MicroRNAs (miRNAs) are short non-coding single stranded RNAs whose classical function is to silence specific genes, acting on a post-transcriptional level. MicroRNAs can also act as ligands for receptors, such as toll-like receptors (TLRs). TLRs belong to the family of pattern recognition receptors and are mainly expressed by innate immune cells including microglia and macrophages. Microglia and invading macrophages accumulate within and around glioma, highly malignant brain tumors with a poor prognosis and survival time for patients. Although TLRs on glioma-associated microglia/macrophages (GAMs) contribute to tumor expansion, and miRNA expression is frequently dysregulated in tumors, the role of miRNAs as signaling molecules in glioma is unknown. Thus, this project aims at investigating the interaction between specific miRNAs and TLRs expressed by GAMs. In the present study we have focused on the let-7 miRNA family, which is abundantly expressed in the brain. We determined miRNA expression levels in normal brain and glioma tissue derived from human and mouse by using TaqMan PCR. The let-7 expression was downregulated in both human and murine glioma samples compared to the control group. Extracellularly delivered let-7 miRNAs activated TLR signaling pathways in microglia in vitro, resulting in the release of inflammatory molecules. We identified let-7a, let-7b, let-7c, let-7e, and let-7g as activators for TLR7 resulting in the release of the cytokines TNFα, IL-6, IL-1β, and IL-10 and the chemokines GRO-alpha, MIP-2 and RANTES. GAMs from experimental mouse glioma revealed an enhanced inflammatory response compared to naïve microglia. Taken together, our data indicate that specific let-7 miRNAs function as extracellular signaling activators of TLR7, thereby driving a pro-inflammatory response in naïve microglia and GAMs.

CELLS WITH CHARACTERISTICS OF CANCER ASSOCIATED FIBROBLASTS IN GBM AND THEIR EFFECT ON MIGRATION OF ENDOTHELIAL AND GLIOMA CELLS

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Glioblastomas (GBM) are the most frequent primary brain tumors in adults characterized by highly infiltrative growth and pronounced neovascularization. Interactions among the cells present in the microenvironment of these tumors may importantly contribute to these processes. Our previous work demonstrated increased expression of the "fibroblast activation protein" (FAP, seprase), a protease typically expressed in cancer associated fibroblasts in carcinomas, in transformed as well as stromal cells in GBM. The aim of this work was to isolate the FAP+ stromal cells from GBM and to determine their effect on endothelial and glioma cells. Glioblastomas contained perivascularly localized FAP+ stromal cells, which characteristically expressed mesenchymal markers SMA, TEM-1 and the fibroblast marker TE-7. Using immunomagnetic separation, we isolated the FAP+ cell from several GBMs and propagated them in the pericyte medium. These cells preserved the expression of mesenchymal markers as determined by immunocytochemistry. In contrast, the expression of glial and endothelial markers was low, which supported the mesenchymal origin of these cultures. Using conditioned media, we demonstrated that these mesenchymal cultures were chemotactically attracted by endothelial cells and at the same time enhanced the migration of endothelial cells. Conditioned media from the FAP+ mesenchymal cultures also promoted the migration of U87 and U251 glioma cells and slightly increased their growth. Taken together, our data demonstrate that FAP+ mesenchymal cells analogous to cancer associated fibroblasts present in carcinomas participate on soluble factor mediated multidirectional interactions among cell subpopulations in human glioblastomas. These FAP+ stromal cells may contribute to the invasive growth and neoangiogenesis in glioblastoma.

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FREQUENT CHROMATIN REMODELING ATRX AND DAXX GENE ALTERATIONS IN THE ABSENCE OF DRIVER IDH OR H3.3 MUTATIONS IN GIANT CELL GLIOBLASTOMAS

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Giant cell glioblastoma (GBM) is a rare histological variant of GBM characterized by the presence of 'numerous' multinucleated giant cells. The lack of a more precise definition of this morphological variant makes that sometimes the giant cell component is not taken into account, even though some evidences may suggest that it may be clinically relevant. Similarly to other GBMs, IDH1 and IDH2 (IDH) mutations are infrequent in giant cell GBMs, but there is scarce molecular data supporting the morphological entity. The purpose of our study is to determine whether there is a molecular genetic base underlying the giant cell morphological variant of GBM. We retrospectively studied 19 cases of giant cell GBMs or GBMs in which a giant cell component was specified in the pathological report. Mean age of the patients was 42 years. We performed next generation sequencing mutational and copy number variation analyses by using a targeted panel of genes involved in gliomas. The results showed that 79% of the cases presented TP53 mutations, a comparable frequency to that of secondary GBMs or lower grade gliomas. Strikingly, 47% of the cases presented mutations in ATRX or DAXX in absence of the previously reported driver IDH or H3.3 gene mutations. Our findings here show that mutations of TP53, as well as alterations in the chromatin remodeling genes, ATRX and DAXX, are frequent in giant cell glioblastoma, while IDH or EGFR mutations are almost absent. Our results support the identification of a subgroup of IDH- H3.3-wild type GBM patients with mutations of TP53 and alterations of ATRX or DAXX that are characterized by the presence of giant cells, young age and a relative good prognosis. These patients may be more sensitive to specific radiation and/or chemotherapy agents, which may be important for the management of these patients.

DNA METHYLATION-BASED CLASSIFICATION OF HUMAN CENTRAL NERVOUS SYSTEM TUMORS

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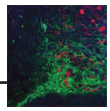

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Background: Modern neuropathology is challenged by an increasing number of clinically-relevant central nervous system (CNS) tumor subgroups that in addition to histopathological analysis currently require assessment of a multitude of molecular markers for classification. Inter-observer variability leads to tumor misclassification, which can have severe consequences for affected patients. Methods: We compiled a cohort of genome-wide DNA methylation profiles of 2,682 tumors from 82 histologically and/or molecularly distinct CNS tumor classes across all ages and histologies that served as reference for a Random Forest-based diagnostic classifier. This classifier was used to prospectively investigate 1,104 CNS tumor samples in order to determine its clinical utility. Reproducibility for different technical platforms and between clinical centers was assessed. Results: The classifier was able to reliably assign CNS tumor samples to a given diagnostic category with a misclassification rate of less than 2%. The system functioned robustly across laboratories and regardless of the method of generating DNA methylation profiles. Prospective application to clinical samples resulted in a reclassification of 12% of tumors with an impact on treatment stratification compared with standard practice alone. Conclusion: This study represents a proof-of-concept for the application of machine learning approaches in molecular diagnostics using a single, easy-to-use assay. The reference cohort and Random Forest-based classifier are available online as a valuable community tool for improving precision in brain tumor diagnostics. We expect that approaches similar to the one presented herein will rapidly restructure diagnostic practice in neurooncology and across tumor pathology.

TRACKING GLIOMA PROGRESSION BY GENETIC BARCODING

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During tumor progression, transformed cells accumulate mutations and gain malignancy. By now, it is unclear how easily this process can be undertaken and if it can be considered the main bottleneck in tumorigenesis. To measure the probability of glioma progression, we used a well-characterized murine model of gliomagenesis, induced by overexpressing PDGF-B in embryonic neural progenitor cells, mimicking a possible first hit of gliomagenesis. In order to univocally tag each PDGF-transduced cell we added to PDGF-B transducing vector a degenerated barcode sequence, and produced high complexity libraries of barcoded retroviruses that were injected in mouse embryos. After the development of gliomas, tumor masses were harvested and analyzed by NGS. By using in-house developed software, we successfully retrieved barcodes from tumor masses and reconstructed the clonal composition of several independent tumors. Analyzed gliomas resulted composed by about one thousand independent clones each. The analysis allowed accounting the diverse size of the clones and estimating their contribution to the whole tumors. Intrestingly, the ten largest clones represented about 50% of the tumor mass. Our results show that tumor masses are composed by cells that do not share a common ancestor, suggesting that the earlier stages of progression do not constitute strong bottlenecks for the tumorigenesis.

CELLULAR AND MOLECULAR IDENTITY OF TUMOR-ASSOCIATED MACROPHAGES IN GLIOBLASTOMA

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Emory University; MDC Berlin; Washington University

Glioblastoma (GBM) is the most aggressive and common type of brain tumor in adults, with patient survival times of approximately one year following diagnosis. The GBM microenvironment is composed of numerous non-neoplastic cells, including vascular endothelia, various infiltrating and resident immune cells, and non-neoplastic glial cells. The most abundant non-neoplastic cell population in the GBM microenvironment is tumor-associated macrophages (TAMs). TAMs comprise mixed populations of myeloid cells, including infiltrating macrophages from the blood circulation and resident brain microglia. TAMs are recruited to the GBM microenvironment, where they are hypothesized to perform immunosuppressive functions and release growth factors and cytokines in response to factors produced by neoplastic cells. In an effort to delineate the temporal and spatial dynamics of TAM composition during malignant gliomagenesis, we employed genetically-engineered mouse models of PDGF-driven GBM in combination with double-transgenic reporter mice that express GFP (green fluorescent protein) or RFP (red fluorescent protein) under the control of CX3CR1 or CCR2 promoters, respectively. Using this approach, we demonstrated that CX3CR1LoCCR2Hi monocytes are recruited to the GBM, where they transition in situ to CX3CR1HiCCR2Lo macrophages and CX3CR1HiCCR2- microglia-like cells. We found that infiltrating macrophages constitute ~80% of the total TAM population, with resident microglia accounting for the remaining ~20% of TAMs. Bone marrow derived infiltrating macrophages/monocytes were recruited to the tumor early during GBM initiation, where they localized preferentially to perivascular areas. In contrast, resident microglia are mainly localized to peritumoral regions. RNA-sequencing analyses revealed differential gene expression patterns unique to infiltrating and resident cells, suggesting unique functions for each TAM population. Notably, limiting monocyte infiltration via Ccl2 genetic ablation prolonged the survival of tumor-bearing mice. Our findings illuminate the unique composition and functions of infiltrating and resident myeloid cells in GBM, establishing a rationale to target infiltrating cells in this neoplasm.

EXPLORING GLIOMA HETEROGENEITY USING IMMUNOBLOTTING AND IMMUNOCYTOCHEMISTRY

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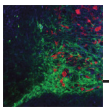
Malignant gliomas are the most common primary brain tumors in adults. Despite advances in neurosurgery, radio- and chemotherapy, the median survival for the most aggressive tumor, glioblastoma (GBM) remains less than 2 years. Major molecular subtypes (classical, proneural, mesenchymal, and neural) were defined that are associated with particular signaling pathways and differential patient survival. Recent large-scale genomic, transcriptomic and epigenetic analyses have provided strong evidence for cellular and molecular



heterogeneity of GBM. Our aim was to elucidate whether patient-derived primary glioma cell cultures recapitulate tumor heterogeneity. For this purpose, we have generated 14 glioma cell lines from freshly operated human tumor specimens of low- and high-grade gliomas and analyzed two glioblastoma stem-like cell lines (generated by Dr. Rossella Galli). All samples were analyzed using immunoblotting and immunocytochemistry, with a reference to certain markers of Verhaak's classification for immunohistochemical analysis. Based on proneural (OLIG2, IDH1-R132H, p53 and PDGFRA), classical (EGFR, and NESTIN) and mesenchymal markers (YKL-40, CD44), as well as high GFAP, MAP2, beta-Tubulin III, we incorporated the results into the transcriptional analysis. These protein-based analyses revealed the striking intratumoral heterogeneity of primary glioma cell cultures, recapitulating tumor complexity. We show subtyping of gliomas of various WHO grades using immunoblotting and immunocytochemistry. However, the mixed expression of subtype specific markers was also seen in the western blot analysis.

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RADIOPROTECTIVE ROLE OF MKP-1 IN GLIOBLASTOMA CELLS IN RESPONSE TO A CXCL12-STIMULATION PRODUCED IN THE SUBVENTRICULAR ZONES.

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We previously described that the subventricular zone (SVZ), one of the two neurogenic regions of the adult brain, could host specifically Glioblastoma-stem cells (GSC) and protect them from irradiation. We showed also that the production of the chemokine CXCL12 in this zone attracts GSC and radioprotects them. Recent works in the lab have also shown that CXCL12 binding to CXCR4 triggers the phosphorylation and therefore the activation of the MAP kinase phosphatase, MKP-1. e observed that MKP-1 is expressed in various GBM cell types including primary cells cultivated from resected human brain tumors and that its phosphorylation level was significantly increased after a CXCL12-stimulation. MKP-1 inhibition does not affect the migratory response of GBM cells to a CXCL12-stimulation. However, in culture, GBM cells invalidated for MKP-1 form significantly less colonies after irradiation. In addition, MKP1 is implicated in the dephosphorylation of the histone H3, helping the cells to repair DNA strand breaks after irradiation in GBM cells. In parallel, we showed that MKP1 is able to dephosphorylate JNK MAP kinases (associated to the apoptosis and cellular stress signals) upon CXCL12-stimulation in GBM cells. The inhibition of this apoptotic pathway could have a major impact on survival after irradiation. In conclusion, we have shown that MKP-1 is highly expressed in GBM cells and that its phosphorylation is increased following CXCL12-stimulation. MKP-1 isn't involved in GBM cells migration but rather in the radioprotection brought by CXCL12 released in the SVZ.

RADIO-INDUCED TRANSDIFFERENTIATION OF GLIOBLASTOMA STEM CELLS INTO ENDOTHELIAL CELLS

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Glioblastomas (GBM) are brain tumors which display a bad prognosis despite conventional treatment associating surgical resection and subsequent radio-chemotherapy. Indeed, these invasive tumors recur almost inevitably. The presence of a radioresistant and tumorigenic GBM Stem-like Cells (GSC) subpopulation could contribute to explain this clinical impasse and the recurrence of these tumors. Furthermore, GBM are characterized by an important and abnormal vascularization and it has been shown that GSC could transdifferentiate into Tumor Derived Endothelial Cells (TDEC)1,2 which are key compounds of tumor growth. We hypothesized that ionizing radiation is able to facilitate transdifferentiation of GSC into TDEC. TDEC appearing within the irradiation field could thus participate to the formation of new vessels and could help remaining tumor cells to develop a new aggressive tumor. We used GSC primocultures from several patients to cultivate irradiated or non-irradiated GSC under endothelial condition to obtain TDEC. Although irradiation did not influence endothelial phenotype of TDEC, we showed that the endothelial functions of TDEC obtained from irradiated GSC were improved (formation of pseudotubes in Matrigel™ and migration towards VEGF). Altogether these results suggest a new mechanism potentially involved in radioresistance of GBM. We are currently trying to decipher which signaling pathway(s) could be involved in this radiation-induced glio-endothelial transdifferenciation. In the future, these signaling pathways could be inhibited in order to decrease or even abolish GBM recurrence.

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AN EFFICIENT AND SCALABLE CRISPR/CAS9 PIPELINE FOR EPITOPE TAGGING IN NEURAL AND GLIOMA STEM CELLS

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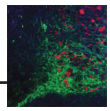
The simple design and efficacy of CRISPR-Cas9 technology has accelerated genome editing at an unprecedented scale across multiple organisms and cell types. Knock-in of small epitope tags into endogenous genes simplifies antibody-based assays, overcoming issues of specificity and sensitivity. Here, we demonstrate highly efficient and scalable CRISPR/Cas9-assisted epitope knock-in using mouse and human primary neural stem (NS) cells and glioblastoma tumour-derived cultures. Three different methods of Cas9 delivery were tested: 1) transient expression through a plasmid, 2) recombinant Cas9 (rCas9) protein transfection, and 3) constitutive expression of Cas9 in cells. We find that the rCas9 protein delivery facilitates efficient knockin of V5 tag (5-10%) without requirements for any selection strategy. Delivery of ribonucleoprotein complexes containing synthetic dual-guide RNA (crRNA 36-mer and tracrRNAs 67-mer) provided even further gains in efficiency, with knock-in efficiencies increase to 5-30% depending on target genes. Similar efficiencies were achieved in mouse and human NS and glioma stem cells. Importantly, with these optimized conditions and a newly developed web-based tool for crRNA and donor DNA design, we were able to demonstrate medium throughput epitope tagging in a 96-well plate format. 192 transcription factors (key regulators of neural stem cell self-renewal and differentiation) were tested for tagging in parallel, and 60 of these were effectively tagged with V5. Our method provides a step-change in our ability to interrogate mammalian proteins in stem cells and their glioma counterparts. As a proof-of –principle, we used the newly tagged glioma cell lines for Sox3-V5 and performed ChIP-SICAP (selective isolation of chromatin associated proteins). In summary we have developed a highly efficient and scalable pipeline for tagging of endogenous proteins in mouse and human neural and glioma stem cells.



ENDOTHELIAL CELL-DERIVED ANGIOPOIETIN-2 IS A THERAPEUTIC TARGET IN TREATMENT-NAIVE AND BEVACIZUMAB-RESISTANT GLIOBLASTOMA

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Objective: Glioblastoma multiforme (GBM) is treated by surgical resection followed by radiochemotherapy. Bevacizumab is commonly deployed for anti-angiogenic therapy of recurrent GBM, however innate immune cells have been identified as instigators of resistance to bevacizumab treatment. Results: We identified angiopoietin-2 (Ang-2) as a potential target in both, naive and bevacizumab-treated glioblastoma. Ang-2 expression was absent in normal human brain endothelium, while the highest Ang-2 levels were observed in bevacizumab-treated GBM. In a murine GBM model, VEGF-blockade resulted in endothelial upregulation of Ang-2 whereas the combined inhibition of VEGF and Ang-2 lead to extended survival, decreased vascular permeability, depletion of tumor associated macrophages, improved pericyte coverage and increased numbers of intratumoral T-lymphocytes. CD206+ (M2-like) macrophages were identified as potential novel targets following anti-angiogenic therapy. Conclusion: Our findings imply a novel role for endothelial cells in therapy resistance and identify endothelial cell/myeloid cell crosstalk mediated by Ang-2 as a potential resistance mechanism. Therefore, combining VEGF blockade with inhibition of Ang-2 may potentially overcome resistance to bevacizumab therapy.

CARNOSINE INHIBITS THE INFILTRATIVE GROWTH OF GLIOBLASTOMA CELLS

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Carnosine (β-alanine-L-histidine) reduces the growth of glioblastoma cells. Here, we investigated its anti-metastatic potential in a co-culture model of fibroblasts and tumor cells derived from patients. Fibroblasts (FB) and primary glioblastoma (GBM) cells were exposed for 48h to carnosine and viability was determined. Co-cultures were created using cloning rings, placing GBM cells inside and patient derived FBs outside the ring and cultures were incubated in the absence or presence of carnosine for 3 weeks. Colony formation and area occupancies of FBs and GBM cells were analyzed by microscopy and quantified using ImageJ. Both, FBs and GBM cells, respond to increased concentrations of carnosine with a reduced production of ATP although the effect was less pronounced in FBs (50 mM: FB: 95.3±5.4%; GBM: 88.3±8.2%; 75 mM: FB: 85.1±5.8%; GBM: 71.3±6.8%). A comparable observation was made by measuring dehydrogenase activities (50 mM: FB: 87.9±4.4%; GBM: 81.2±7.1%; 75 mM: FB: 78.8±5.0%; GBM: 61.8±4.8%). Co-culture experiments revealed that carnosine strongly inhibited the formation of tumor cell colonies within the fibroblast layer (85.8±50.5 in the absence of carnosine) compared to 46.6±26.4 (10 mM), 28.8±4.0 (25 mM) and 1.25±0.5 (50 mM). Furthermore, the area covered by tumor cells was reduced from 13.4±3.9% (no carnosine) to 7.7±2.5% (10 mM), 6.0±3% (25 mM) and 3.1±3.2% (50 mM). Although viability of FBs was reduced by carnosine the effect on GBM cells was more pronounced. More importantly, the dipeptide significantly inhibited colony formation and migration in a fibroblast co-culture model leading to a reduced number of tumor cell colonies without affecting fibroblast growth even at a concentration of 10 mM carnosine. Therefore, we assume carnosine may strongly inhibit the occurrence of recurrent tumors preventing the outspread of tumor cells into surrounding tissue.

TUMOUR MASS VERSUS SUBVENTRICULAR ZONE: MOLECULAR AND FUNCTIONAL CHARACTERISATION

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Life expectancy after diagnosis of glioblastoma (GBM) remains poor even with the best available treatment. This catastrophic survival is the direct consequence of systematic tumour recurrence. It has previously been demonstrated that the subventricular zone (SVZ, a neurogenic niche of the adult central nervous system) attracts and harbours GBM cells, expressing stem cell biomarkers that act like glioblastoma-initiating cells. These cells hosted in the SVZ may be responsible for tumour recurrence. To better understand the difference between cells from the tumour mass (TM) and those migrating to the SVZ, this project will focus on two axes. First, a proteomic and transcriptomic approach will be used to identify one or more biomarkers that might help to establish if recurrences originate from the TM or the SVZ. The second aim of the project is to compare these two populations of cells from a functional point of view. Some functional differences have already been observed: cells derived from SVZ form more spheroids than those derived from TM (1). U87MG cells from SVZ also form tumours more easily in mice compared to cells derived from the TM (2). However, preliminary results in chemo- and radioresistance assays on the cultured cells (U87MG) do not show significant differences between the two populations. Therefore, it might be the SVZ environment that plays a key role in therapeutic resistance, rather than intrinsic biological differences of cells from the TM versus the SVZ.

1. Goffart N et al. doi:10.1093/neuonc/nou144. 2015.

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ROLE OF ACYL-COA BINDING PROTEIN (ACBP) IN GLIOBLASTOMA MULTIFORME TUMOR INITIATION AND DEVELOPMENT

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Glioblastoma Multiforme (GBM) is a devastating form of aggressive brain cancer with very grim survival outlook after initial diagnosis (~14 months). Our previous research has shown that under physiological conditions in mice adult neurogenic niches, ACBP expression in neural stem cells regulates their proliferative state. Clinical data from GBM patients show overexpression of ACBP in these tumors and a worse survival rate in patients with strong ACBP expression. Here in our study we show that both in vitro and in vivo, lack of ACBP severely reduces the proliferation of GBM tumor cells, and delays and slows down the development of tumors in vivo without eliciting an apoptotic response. Our pharmacological experiments indicate that lack of ACBP likely interferes with the transport of Long Chain Fatty Acyl-CoA (LCACoA) into the mitochondria via CPT1, therefore depleting tumor cells from a major energy supply. Based on our results we propose ACBP as a putative target for manipulating GBM tumor metabolism and subsequent prevention of rapid tumor cell proliferation.

THE MUCIN-LIKE GLYCOPROTEIN PODOPLANIN IN GLIOBLASTOMA

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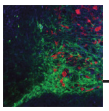

The transmembrane protein podoplanin (PDPN) is expressed in various organs including the brain where it is restricted to the choroid plexus, ependymal and neural stem cells. In primary glioblastoma (GB) strong PDPN expression, due to loss of the PTEN tumor suppressor, correlates with shorter overall survival. Consistently, gene set enrichment analyses and serial xenotransplantation of patient-derived GB cells supported the correlation of high PDPN expression and malignancy. To decipher the functional role of PDPN on tumor progression we deleted PDPN in these cells using the CRISPR/Cas9 technology. However, we do neither observe a significant survival benefit in recipients injected with PDPNKO tumor cells nor do these tumors display an altered histology compared to control tumors. Taken together, our current in vivo and complementary in vitro data support the notion that PDPN is a valuable marker for poor prognosis but does not constitute a major driver for malignant progression of GB. Interestingly, in the course of our studies, we detected Pdpn expression not only in tumor cells, but also in the tumor microenvironment, raising the question whether this might cause the poor prognosis for GB patients exhibiting high PDPN levels. Thus, we first sought to determine the source of Pdpn expression in the microenvironment. Employing stainings of tumor sections and primary cell isolations, we found Pdpn expression in tumor associated-myeloid cells and reactive astrocytes. Currently we are engaging mouse models that harbor a specific Pdpn loss in these cells as recipients for orthotopic tumor cell transplantations to elucidate the impact of microenvironmental Pdpn on GB progression.

EXPRESSION AND FUNCTION OF NFAT (NUCLEAR FACTOR OF ACTIVATED T CELLS) TRANSCRIPTION FACTORS IN HUMAN GLIOMAS

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Brain macrophages (microglia) and peripheral macrophages accumulate in malignant gliomas and are re-programmed into tumor supportive cells that enhance invasion and induce immunosuppression. We found that GM-CSF (granulocyte macrophage colony stimulating factor) is a crucial driver of microglia/macrophage accumulation in experimental gliomas, in contrast to non-CNS tumors, in which M-CSF (macrophage colony stimulating factor) attracts macrophages into tumor. Our studies suggest that the expression of Csf-2 (coding for GM-CSF) in gliomas is regulated by abnormally activated NFAT (Nuclear Factor of Activated T cells) factors. Initially discovered in T cells, the NFAT family, composed of four calcium-responsive members, is regaining attention due to its contribution in carcinogenesis. Our analysis of TCGA data revealed significant upregulation of NFATc1, NFATc2 and NFATc3 in human grade II-IV gliomas. We also evaluated the expression of different members of NFAT family using qPCR in human low and high grade gliomas as well as in established and primary glioma cell lines in comparison to non-tumoral brain samples and normal human astrocytes. Chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq), was used to identify new transcriptional targets of NFAT in glioma cells. The results suggest that NFAT proteins may control a new program of cytokine/chemokine expression, which is important for glioma-microglia communication and glioma progression, and thus constitute an plausible target for future therapeutics.

The study was supported by grant 2014/15/B/NZ3/04704 from The National Science Centre, Poland.

ACTIVATING TRANSCRIPTION FACTOR 5: A NEW THERAPEUTIC TARGET IN ASTROCYTOMAS OF DIFFERENT WHO-GRADES AND VARYING BIOLOGICAL BEHAVIOUR?

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Activating transcription factor 5 (ATF5), a basic leucine zipper protein, suppresses differentiation of neuroprogenitor cells into glia or neurons in the normal brain (NB). It is overexpressed in glioblastoma (GBM). A reduction of its expression or activity leads to p53-independent apoptotic GBM-cell death, therefore suggesting that it could serve as a novel therapeutic target. To our knowledge, no data on ATF5 expression in low grade astrocytomas (LGA) or recurrent GBM has been published so far. ATF5 mRNA-expression of frozen patients' samples was measured by duplex qPCR. Overexpression of ATF5 mRNA was observed in LGA (7 fold, $p<0.001$, n=40) and GBM (10 fold, $p<0.001$, n=79) compared to NB (n=10). In addition, the panel of GBM was grouped according to varying biological behavior, i.e. local/multifocal growth or primary tumor/relapse, tumor localization and age at primary diagnosis, showing no significant difference between the analyzed groups ($p>0.05$). GBM patients were then allocated by the median ATF5 overexpression compared to NB (6 fold) to a „low ATF5 expression" and a „high ATF5 expression" group. Kaplan-Meier analysis and cox regression indicated that ATF5 mRNA expression does not significantly correlate with long term ($p=0.084$, median survival 18.9 vs. 13.7 months), but with short term ($t<12$ months, $p=0.022$, HR 2.827) and progression free survival (12.8 vs. 6.9 months, $p=0.024$). Since ATF5 is significantly overexpressed on mRNA level in LGA and GBM independently of tumor growth patterns and its inhibition might lead to the selective death of glioma cells, but not of non-tumor cells, it might serve as a potential ubiquitous therapeutic target in astrocytic tumors.

MOLECULAR AND CYTO-ARCHITECTONIC RESHAPING OF THE HUMAN SVZ DURING GLIOMA INVASION

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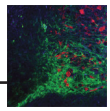
The sub-ventricular zone (SVZ) lining the ventricles is a specialized niche for Neural Stem Cells (NSCs). Clinical studies have shown that Glioma patients with tumors contacting the SVZ have earlier recurrences and overall worse prognosis than non-SVZ-contacting tumors. Molecular and cellular events regulating Glioma homing to the SVZ are largely unknown. Here, we have compared normal (N=5) and high-grade Glioma samples (N=20) of the human SVZ by Immunofluorescence and volumetric image analysis. Glioma samples showed gradual stages of invasion; SVZ cytoarchitecture varied from relatively normal (low-invasion) to completely disrupted (high-invasion). Stainings for Laminin and Fibronectin revealed upregulation of vascular-associated extracellular fractones, size and density of which increased up to 3 fold in low-invasion Glioma samples compared to controls. In contrast, high-invasion samples showed disappearance of the fractones network in favor of local neo-vascularization. Analysis of the normal SVZ by qPCR revealed local enrichment of the chemokine SDF1α, ligand for the CXCR4 receptor and potent in vitro chemoattractor of Glioma Stem Cells. Histology revealed SDF1α expression specifically in the ependymal layer. CXCR4 was enriched in the NSCs-astrocyte ribbon, as well as present in Microglia. In low-invasion Glioma samples, ependymal showed a 3-fold upregulation of SDF1α. Density of Microglia was proportional to SDF1α expression. Accordingly, CXCR4 expression was widely increased in the neighboring parenchyma, including in the tumor cell population. Our study highlights an active reshaping of the ependymal, and the hypocellular layers of the human SVZ during Glioma invasion. Further, our data indicate that the SDF1α-CXCR4 signaling axis is involved in Glioma homing to the human SVZ, supported by previous observations in rodent models.



NKG2D LIGANDS IN GLIOMA STEM-LIKE CELLS: EXPRESSION IN SITU AND IN VITRO

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Glioblastoma multiforme (GBM) is a highly malignant brain tumor. Tumor stem cells have a major influence on tumor malignancy, and immunological escape mechanisms, involving the Natural Killer Group 2, member D (NKG2D) receptor-ligand system, are key elements in tumor immunosurveillance. We analyzed the expression profile and localization of NKG2DL and embryonic and neural stem cell markers in solid human GBM and stem-like cells isolated from glioma cell lines. We also evaluated the effect of temozolomide (TMZ) on NKG2DL expression. Whereas Musashi-1 and Oct-4 were rarely costained with NKG2DL in two colour immunostaining, Sox-2 and Nanog showed partial costaining and Klf-4 complete costaining with NKG2DL. NKG2DL were found in a distinct tumor cell subpopulation and were broadly costained with each other, though single positive cells were found as well. qRT-PCR and immunostaining showed, that stem-like cells derived from T98G were predominantly positive for MICB and Klf-4, whereas in U251MG MICA, ULBP2, Sox-2, Nanog and Musashi-1 were more pronounced. With differentiation, T98G displayed significantly less NKG2DL, whereas in U251MG expression of most stem cell markers decreased. Stimulation with TMZ led to a significant upregulation of NKG2DL. The role of the NKG2D system in glioma stem cells is complex. As stem-like glioma cells show a higher expression of NKG2DL than more differentiated tumor cells and TMZ treatment supports upregulation of NKG2DL, the NKG2D system might play an important role in tumor stem cell survival and in GBM therapy.

FUNCTIONAL CHARACTERIZATION OF ONCOGENIC-INDUCED CELL PLASTICITY IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and lethal form of intracranial tumor. Using a Cre-inducible lentiviral GBM mouse model we recently showed that gliomas can originate from terminally differentiated neurons and astrocytes, which can dedifferentiate to a stem cell-like state upon transformation. In this study, we performed whole transcriptome analysis and confirmed that transformed dedifferentiated astrocytes and neurons acquired a stem/progenitor cell state, although they still retained gene expression memory from their parental cell. Functional analysis of the transcriptomics data revealed involvement of the Wnt signaling, cell cycle and the focal adhesion pathways in defining the state of the dedifferentiated cell-types. Our analysis further revealed conservation of a gene interaction network in both dedifferentiated cell-types. This network exhibited a modular architecture, connecting components of the cell cycle pathway to Wnt signaling and the focal adhesion pathways, with the gene Spp1, also known as osteopontin (OPN) serving as a key common node connecting these three pathways. Specific inhibition of OPN in both murine and human glioma tumors prolonged mice survival. We are currently validating the expression of additional interacting partners. We believe that genetic perturbation of key players and/or the abolishment of their interactions can help elucidate the regulatory mechanism of this network in maintaining the dedifferentiated state of the transformed neurons and astrocytes.

APELIN FUNCTION IN ENDOTHELIAL/TUMOR INTERACTION IN GLIOBLASTOMA

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Background: Glioblastoma (GBM) are aggressive brain tumors that contain a subpopulation of highly plastic self-renewing cancer cells, also named glioblastoma-initiating cells (GIC). Glioblastoma recurrence and resistance to therapy is closely associated with GIC that reside in vascular niches within the tumor. These GIC are regulated by external cues emanating from endothelial cells, however the nature of such angiocrine signals remains unknown. Methods: We used a well-characterized panel of patient-derived cells to assess the effects of the endothelial secreted factor apelin on self-renewal using standard stem cell assays. Extinction strategy was designed to assess the significance of apelin signaling in GIC tumorigenicity. Additionally, we employed in vitro pharmacological assays and functional tests, as well as in vivo toxicity tests to describe the competitive APJ antagonist MM54. Furthermore, we implemented ectopic and orthotopic mouse models of GIC to test the efficacy of MM54 on tumor growth in vivo. Results: Here, we deployed a proteomic approach to characterize factors released by brain endothelial cells and identified apelin. Silencing and pharmacological targeting of the apelin receptor APJ inhibited endothelial-mediated pro-self-renewal effects ex vivo and impaired tumor growth in mice. Functionally, selective competitive antagonists of APJ were shown to be safe and effective in vivo, and to significantly lengthen survival in intracranially xenografted mice. Conclusion(s): Our results demonstrate that the apelin/APJ signaling nexus operates to sustain GIC. Given the concerns about current chemotherapy regimen, including resistance, targeting apelin provides new opportunities for future therapeutics in the treatment of these aggressive tumors.

GLUTAMATE METABOLISM AND INFLAMMATORY PROFILE OF TUMOR ASSOCIATED MICROGLIA/MACROPHAGES

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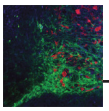
Dysregulation of glutamate signaling is a hallmark of glioblastoma (GBM) and various neurodegenerative diseases. GBM cells secrete high levels of glutamate (Glu) especially through the upregulation of System Xc, a glutamate/cystine antiporter. Glu triggers neuronal cell death and subsequently provides more space for tumor cells to invade and expand. Tumor-associated microglia/macrophages (TAMs), consisting of brain resident microglia and monocyte-derived macrophages (MDMs) recruited from the blood, largely contribute to the tumor mass and exhibit an alternative, tumor-supporting phenotype. We reported transcriptional changes in glutamatergic signaling of TAMs after in vitro exposure to GBM cells. A comparative analysis of freshly-extracted TAMs, cultured TAMs and MDMs exposed to glioblastoma cells indicated disparate profiles among these cell types, thus strengthening the concept of functional differences



between TAMs and MDMs. Following on this qPCR study, we want to investigate the cross-talk between Glu signaling in TAMs and MDMs and their inflammatory status to gain more information on the specific function of these cell types. For that purpose, TAMs and MDMs untreated or treated with pro- or anti-inflammatory stimuli are exposed for short time periods (6h, 24h) to either normal human astrocytes or GBM cells. Following indirect co-culture, TAMs and MDMs are analyzed for their Glu signaling and inflammatory status at the gene and protein expression levels by qPCR, flow cytometry and immunoblotting. The inflammatory status is further defined by analyzing the expression of specific micro-RNAs. The metabolome of the culture medium is examined using mass spectrometry. Data obtained from the analysis of MDMs will be reported and discussed at the Brain Tumor meeting.

A MICROFLUIDIC SYSTEM FOR GBM PROGRESSION STUDIES

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Over the years, many in vitro and in vivo models have been used to study different aspects of malignant tumours growth. As currently known, developing a system to represent a complicated microenvironment, which often plays a key role in cancer progression, is one of the biggest challenges that brings together scientists specializing in biology, engineering, physics and many more. In the organ-on-chip technology field, one possible application of microfluidic devices is in mimicking the tumour microenvironment. Microfluidic devices are most commonly made using polydimethylsiloxane (PDMS) because of the many desirable properties of this material (i.e. non-cytotoxic, gas permeable, flexible). Recent studies have also started incorporating hydrogels inside the devices as scaffold for 3D cell culture and support for vascular networks. Here, we present a microfluidic system which can be modified and used for a variety of GBM progression related studies. Multiple channels and compartments within the device allow us to create an in vitro platform mimicking niches where cells could be triggered to change their phenotype, what we believe is crucial in GBM recurrences. We have established a system consisting of an endothelial barrier layer (mimicking the blood brain barrier) separating a fluidic channel (for oxygen and nutrient delivery/disposal) and a gel extracellular matrix (ECM). Different GBM cell populations can be embedded in extracellular matrix (ECM), which is proximal to the endothelial layer and furthermore, any changes in cancer cell proliferation and migration are monitored by optical microscopy. Last but not least, we are optimizing the system for cell extraction. Thus, after appropriate time in 3D culture (depending on an experiment) we will be able to purify RNA and subject to analysis.

OPPOSITE ROLES OF NOTCH SIGNALING IN THE FORMATION OF DISTINCT GLIOMA SUBTYPES

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Neural stem cells in the postnatal brain are believed to be one origin of brain tumors such as gliomas. The Notch signaling pathway is required for neural stem cell maintenance and, accordingly, promotes a self-renewing stem cell-like state in glioma cells. Therefore, Notch signaling is believed to be oncogenic in glioma, primarily by virtue of its stem cell promoting activity. However, inactivating mutations in Notch pathway components and low Notch signaling activity have been identified in glioma subtypes in humans, suggesting a tumor suppressive role. We addressed the role of Notch signaling in glioma formation using conditional genetics and lineage tracing in mouse models of human brain tumors and discovered a context dependent function for the Notch pathway in distinct glioma subtypes. We found that gliomas driven by either p53 loss of function or Akt gain of function can originate from a cell population with high Notch signaling activity. However, surprisingly, Notch has opposite functions in these glioma subtypes. Genetic deletion of core Notch pathway components accelerates growth of gliomas driven by p53 loss of function and, conversely, genetic activation of the Notch pathway reduces glioma formation. In stark contrast, genetic deletion of Notch pathway components delays the development of gliomas driven by gain of Akt signaling. Interestingly, individual Notch receptors have distinct functions during glioma development, and only specific Notch receptors or receptor combinations can activate a tumor suppressive signal. Hence, Notch receptor paralog and glioma subtype dictate the tumor suppressive versus oncogenic role of Notch signaling.

THE IMPACT OF IDH1/2 MUTATIONS ON SOCS GENE METHYLATION AND DEREGULATION OF STAT SIGNALING IN GLIOMAS

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DNA methylation is frequently deregulated in tumors, however it is not clear if this a cause or consequence of tumor transformation. Gliomas, the most common tumors of the central nervous system, represent over 70% of all brain malignancies. Many gliomas have deregulated DNA methylation and some exhibit the hypermethylation phenotype. Whole genome sequencing revealed mutations in the genes coding for metabolic enzymes IDH1/2 that result in generation of an oncometabolite blocking epigenetic enzymes. Some of events (i.e. methylation of the MGMT promoter which silences a gene of DNA repair) are beneficial, while other could be deleterious. We performed an analysis of the occurrence of IDH1/2 mutations by PCR and Sanger sequencing in a panel of 58 low and high grade gliomas and studied methylation of the SOCSs genes coding for negative regulators of cytokine/growth factors signaling. These signaling pathways converging on STAT transcription factors are frequently deregulated in gliomas and contribute to tumorigenesis. The analysis showed correlation between the presence of IDH1 mutation and methylation of SOCS1 and SOCS3 (SOCS2 methylation did not correlate with IDH1/2 status). Methylation of the MGMT gene has been studied in the same samples, as the confirmation of the hypermethylator phenotype. Our results would help to establish a risk group that is particularly vulnerable to glioma development among low grade tumors. Detection of mutations in the IDH1/IDH2 genes and SOCS1, SOCS2 and SOCS3 genes methylation could be a simple diagnostic test allowing classification of patients with increased risk transformation to malignant tumor.

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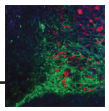

MINOCYCLINE REDUCES PRODUCTION OF TUMOR-DERIVED OSTEOPONTIN/SPP1 AND MODULATES THE IMMUNE MICROENVIRONMENT OF RAT C6 GLIOMAS

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Glioma associated microglia and macrophages (GAMs) support tumor invasion and contribute to immunosuppression. We demonstrated a critical role of Osteopontin (Spp1), a potent immune cell attractant and activator, secreted by glioma cells in microglia reprogramming and glioma progression. Minocycline (Mino) has been reported to affect glioma growth via the inhibitory effect on matrix metalloproteinases in microglia or induction of autophagy in glioma cells. We hypothesized that Mino may impair Spp1 expression in glioma cells that would affect responses of immune cells in a tumor microenvironment. We demonstrate that Mino inhibits Spp1 expression in cultured glioma cells. Systemic application of Mino (30 mg/kg b.w) to animals implanted intracranially with C6-Luc+ glioma cells reduced tumor volumes at day 14th as determined using an In Vivo-Imaging Xtreme. Immune heterogeneity of glioma microenvironment was analyses by FACS in controls and Mino treated animals. Expression of selected GAM markers in sorted CD11b+ cells from glioma-bearing hemispheres and cytokine production in glioma-bearing hemispheres were measured. Mino treatment did not affect microglia accumulation, but blocks protumorigenic activation in generated CD11b+ cells and increase expression of selected GAM markers. The increased accumulation of macrophages and leukocyte subpopulations was detected in Mino-treated rats. Profiles of cytokine production in tumor-bearing hemispheres from Mino-treated animals showed differences in the production of pro-inflammatory cytokines and macrophage attractants when compared with controls. We conclude that Mino treatment reduced the expression of pro-invasive factors secreted by glioma cells (i.e. Spp1) that resulted in immunosuppression switch and the increased expression of antitumor response genes in glioma-bearing hemispheres and GAMs. Our results validate Mino treatment as a promising strategy to block Spp1 production and GAM reprogramming.

Supported by 2012/04/A/NZ3/00630 grant from the National Science Center.

MAPK- AND PI3K/MTOR- SIGNALING CASCADE: QUANTITATIVE GENE EXPRESSION ANALYSIS IN HUMAN GLIOMA IN RELATION TO ITS CLINICAL RELEVANCE

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Aims: Human Glioblastomas are one of the most devastating tumors and therefore object of various studies. The MAPK- and PI3K/mTOR signaling cascades are known to be overactivated in various tumors and involved in cell-proliferation, cell growth and abnormal vascularization. Our study analyzes the genetic expression of AKT1, ERK1, ERK2, b-RAF, mTOR, PDGFR-β and VEGFR2, which have a critical role in those pathways. Furthermore the expression was examined in relation to its clinical relevance. Methods: To quantitatively examine the genetic expression with qPCR, the mRNA of 56 surgically obtained high grade glioma samples (WHO°III: n=13, WHO°IV: n=46) was isolated and transcribed to cDNA. Efficiency based ΔΔCt-method against non-pathological tissue as external control was used to analyze the expression rate. We transacted Spearman correlation across all genes and WHO-grades. An established clinical database was used to perform Cox regression analyzes between overall survival and the experimental findings. Results: The mRNA of AKT1, ERK1, ERK2, b-RAF, PDGFR-b was overexpressed in WHO°IV grade samples compared to non-pathological tissue. A significant difference between the WHO-grades was shown only for ERK2 (p=0.02) and b-RAF (P=0.004). WHO°III samples overexpressed only AKT1. In both WHO-grades mTOR was downregulated. There was a significant positive correlation between ERK1, ERK2 and b-RAF, while AKT1 and mTOR showed no correlation. Clinically we conducted a longer mean survival of 7.8 months based on the expression rate of b-RAF (p=0.035) for all patients. Cox regression analysis disclosed a significant hazard ratio (HR) of 1.275 for b-RAF (p=0.005). Conclusions: MAPK-pathway is genetically overly activated in WHO°IV glioma, but not in the WHO°III glioma. The genetic expression rate of b-RAF appears to be of clinical relevance.

STRONG FGFR3 STAINING IS A MARKER FOR FGFR3 FUSIONS IN DIFFUSE GLIOMAS

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Background: Inhibitors of fibroblast growth factor receptors (FGFRs) have recently arisen as a promising treatment option for patients with FGFR alterations. Gene fusions involving FGFR3 and transforming acidic coiled coil 3 (TACC3) have been detected in diffuse gliomas and other malignancies, and fusion-positive cases have responded well to FGFR inhibition. As high FGFR3 expression has been detected in fusion positive tumors, we sought to determine the clinical significance of FGFR3 protein expression level as well as its potential for indicating FGFR3 fusions. Methods: We performed FGFR3 IHC on tissue microarrays containing 676 grade II-IV astrocytomas and 116 grade II-III oligodendroglial tumor specimens. Fifty-one cases were further analyzed using targeted sequencing. Results: Moderate-to-strong FGFR3 staining was detected in gliomas of all grades, was more common in females, and was associated with poor survival in diffuse astrocytomas. Targeted sequencing identified FGFR3-TACC3 fusions and an FGFR3-CAMK2A fusion in 10 of 15 strongly stained cases, whereas no fusions were found in 36 negatively-to-moderately stained cases. Fusion-positive cases were predominantly female and negative for IDH and EGFR/PDGFRA/MET alterations. These and moderately stained cases show lower MIB-1 proliferation index than negatively-to-weakly stained cases. Furthermore, stronger FGFR3 expression was commonly observed in malignant tissue regions of lower cellularity in fusion- negative cases. Importantly, subregional negative FGFR3 staining was also observed in a few fusion-positive cases. Conclusions: Strong FGFR3 protein expression is indicative of FGFR3 fusions and may serve as a clinically applicable predictive marker for FGFR inhibitor-based treatment regimens.

IS EGCG A USEFUL ANTIOXIDANT SUPPLEMENTATION TO FIGHT GLIOMAS?

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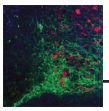

Objective: The cancer-preventive and anti-proliferative effects of the antioxidant epigallocatechin gallate (EGCG) are widely supported by literature. However, effects of antioxidants are concentration dependent and range from beneficial to toxic. There is little information about the effects of EGCG in physiologically reachable concentrations on the progression of glioma cells. This prompted us to examine these effects. Methods: Two primary glioblastoma cell cultures were incubated with EGCG in the physiologically reachable concentration of 100nM for 24hours in total. Viability assays were performed to delineate cell growth. Apoptosis and autophagy were analyzed via Western Blot and quantitative PCR. Results: Incubation with 100nM EGCG did not change cell viability. Further results imply the absence of autophagy and apoptosis initiation: increasing protein expression of the anti-apoptotic protein Bcl-2, an inactive initiator Caspase 8, and a slightly activated effector Caspase 3. The conversion of LC3B and protein ubiquitination did not increase. Transcription of DAPK2, a regulator of apoptotic and autophagic cell death signals with tumor-suppressive functions, increased strongly in both cultures. Conclusion: Our data show that in a physiologically reachable concentration EGCG does not induce cell death in glioblastoma cells. In contrast, as a mild stressor it activates endogenous mechanisms of repair and maintenance to protect cells against subsequent stressing. So, the exposure to EGCG in physiological concentrations leads to a habituation of the cells. Cancer-preventive and anti-proliferative effects of EGCG can only be achieved by application of higher concentrations.

TRANSCRIPTION FACTOR PU.1 IS INVOLVED IN THE PROGRESSION OF GLIOMA

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Glioma is the most prevalent and malignant primary brain tumor with poor prognosis in the central nervous system. The tumor mass does not only consist of glioma cells but also other cell types, like endothelial cells, astrocytes and tumor-associated myeloid cells (TAMs). TAMs include resident microglia and peripheral macrophages and the dual role of TAMs during the glioma progression has been recognized. The differentiation of TAMs is dependent on the transcription factor Pu.1 (also known as Sfpi), it is still unknown whether Pu.1 is involved in the progress of glioma. Thus the study of Pu.1 may help us to uncover the intricate interactions between tumor initiating cells and its microenvironment. We found significant increase of Sfpi, the gene encode Pu.1, in glioma patient samples. Through genotype-phenotype association analysis, we provided several candidate factors that could mediate the role of Pu.1 in glioma. To further validate the association between PU.1 and glioma, we found that the expression of P2XR7, a potential target of Pu.1, is also upregulated in glioma patients. We also showed that various biological pathways could be involved in Pu.1-associated glioma via analyzing these potential targets in the Reactome database. These results provide evidence that Pu.1 could play a role in the progress in glioma through its transcriptional targets in multiple signaling pathways. Thus, in addition to its role in hematopoietic linage development and leukemia, Pu.1 is involved in the regulation of glioma and probably in other malignant carcinomas.

LIVE CELL IMAGING OF TUMOR-ASTROCYTE INTERACTIONS

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Reactive gliosis is a neurodefensive response to CNS challenges, with astrocytes undergoing phenotypic and transcriptional changes aimed at limiting CNS damage. Astrocyte reactivity has been observed in a plethora of neurological disorders, which include inflammatory and non-inflammatory disease, ischaemia, and trauma, and there is evidence that persistence of this process can have adverse effects on the surrounding environment and cells. Furthermore, there is evidence of the involvement of astrogliosis in tumor progression in brain cancers; low grade tumors are cordoned off by glial scars that serve as barriers to invasion, whilst high grade gliomas actively suppress the formation of scars which enables tumor invasion. A better understanding of the effects of astrocyte reactivity on tumor progression is key to unravelling tumor-microenvironmental interactions and developing more targeted therapies. Here we explore the contributions of direct interactions between adjacent cells to astrocyte reactivity. For this, we utilize in vitro co-culture systems to observe and interrogate the underlying mechanisms in real time. In astrocyte/glioma co-culture paradigms, we find that astrocyte polarization precedes changes in cell orientation to form a barrier at the border of the two cell populations. Further work will be carried out to determine whether cell contact-dependent cues guide astrocyte orientation changes. This model will allow the investigation of cell-autonomous or non-cell-autonomous molecular determinants of astrocyte reactivity in the context of glioma. Ultimately, this will allow the identification of tumor-specific negative regulators of gliosis.

HDAC EXPRESSION PATTERN IN GLIOMA-ASSOCIATED MICROGLIA

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While epigenetic dysregulation in glioma has been intensively studied, epigenetic changes within the tumor microenvironment such as glioma-associated microglia (GAMs) have not been in focus. HDAC inhibitors recently entered clinical trials for glioblastoma therapy. Based on their described immunosuppressive effects on microglia it is questionable whether their action on GAMs might be beneficial with regard to anti-glioma therapy, enforcing the need for detailed studies on the role of specific HDAC members in naive as well as tumor-associated microglia. In this study we first characterized HDAC expression levels in microglia. Expression of HDAC1, 3 and 11 was highest in primary and freshly isolated microglia suggesting a prominent role of these HDAC members in microglia. Exposure of primary microglia to glioma-conditioned medium (GCM) significantly increased mRNA expression levels of HDAC1 and 9 while HDAC4 and 7 as well as HDAC class IIb members HDAC6 and 10 were significantly decreased. The upregulation of HDAC1 and the downregulation of HDAC6 and 10 was confirmed in GAMs isolated from the GL261 and RCAS experimental tumor mouse models as compared to healthy controls, classifying them as interesting novel candidates for modulating the GAM phenotype. An additional aim of this study is to identify glioma-derived ligands involved in modulating HDAC expression in GAMs. Toll-like receptors (TLRs) serve as important routes for communication between glioma cells and microglia and the endogenous TLR4 ligand Tenascin C (Tnc) is highly upregulated within the glioma microenvironment. Here we report, that Tnc-stimulation of microglia leads to a significant increase of HDAC1 along with a significant downregulation of HDAC6 and 10 mimicking the impact of the glioma environment on HDAC expression changes. Thus Tnc emerged as a new candidate for inducing the GAM phenotype.

ORGANOTYPIC GLIOBLASTOMA TISSUE SLICES CAN BE ANALYZED BY RNA SEQUENCING, WHOLE SLICE HISTOLOGICAL ANALYSIS AND IMMUNOBLOTTING

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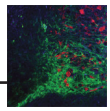

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The individual treatment of brain tumor patients requires experimental models that can be used to predict the outcome of a certain treatment strategy. Today, cell culture or mouse models are often used for the development of test systems, but poorly reflect the tumor's biology. Here, we present that organotypic slice cultures can be used for an automatic analysis of tumor response to treatment. We describe an approach to semi-automatically analyze immunostained slices. We further demonstrate that whole-transcriptome sequencing can be successfully applied to RNA isolated from cultivated tissue slices, as well as protein analysis by immunoblotting. Glioblastoma and normal brain tissue slices were treated by irradiation and temozolomide or left untreated. Caspase-3 and Ki-67 expression within the tissue was determined from whole slices using a newly developed automated scanning technology. Protein was isolated for immunoblot analysis and RNA was processed for whole transcriptome sequencing. Quality control revealed high quality RNA, sequencing libraries could be established and the RNA could be sequenced with a depth of 200 x 106 reads per sample. Principal component analysis of annotated genes revealed great differences between normal and tumor tissue and between treated and untreated samples. 4315 differentially expressed genes were found between untreated tumor and normal brain tissue. Our model offers the opportunity to analyze the response of individual patient-derived glioblastoma tissue to treatment, which we believe is an important step towards personalized therapy of patients.

AUXILIARY EFFECT OF TUMOR-TREATING-FIELDS IN CONJUNCTION WITH MITOTIC CHECKPOINT INHIBITION ON GLIOBLASTOMA CELLS

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Tumor Treating Fields (TTFields) are alternating electric fields with intermediate frequency (100-400kHz) and low intensity (1-3V/cm). They inhibit spindle fiber formation and perturb the division of glioblastoma (GBM) cells. GBM-patients treated with TTFields in addition to the standard therapy showed a significant increase in progression free and overall survival (EF-14 trial) without serious adverse reactions. Monopolar spindle 1 (MPS1), the key regulator of the spindle assembly checkpoint (SAC), guards the fidelity of sister chromatid separation and their distribution to daughter cells. We hypothesized that MPS1 inhibition could enhance the efficacy of TTFields. Human U87 GBM cells were treated with TTFields (Novocure inovitro™ laboratory research system) with or without 4µM MPS1-IN-3 inhibitor (IN3). Cell number and apoptosis (Annexin V staining) were evaluated after 24h, 48h and 72h of treatment and cell growth followed for another 72h after end of treatment (eot). Nuclear anomalies were evaluated by immunefluorescence. Incubation of cells with TTFields and IN3 for 72h reduced the cell number by 77.5% vs. TTFields alone and by 50% compared to sole inhibition with IN3. The growth inhibitory effect of the double treatment emerged earlier and was sustained after eot (78% fewer cells compared to the control). The combination treatment induced an early stage of apoptosis in 44% of the cells, compared to 14% with TTFields alone and 4% with IN3 alone, probably due to nuclear anomalies, which were enhanced by 70% in the combination treatment compared to the single treatments. TTFields are an approved new treatment option for GBM, which could be further improved in their onset, efficiency and sustained effect by a combination with SAC inhibition.

BEHAVIOR OF HUMAN GLIOBLASTOMA CELL LINES IN DIFFERENT HYPOXIC CONDITIONS IN VITRO

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In solid cancers such as glioblastoma (GBM) tissue oxygenation and nutrient supply are heavily disturbed. Tumor cells are exposed to gradients of oxygen and nutrients that shape their capacity for proliferation, invasion and survival. To meet their high energetic needs these cells, contrary to normal cells, rely on aerobic glycolysis to generate ATP in sufficient and insufficient O2 conditions. How this phenomenon, the Warburg effect, supports the high energy demand of tumor cells is not yet fully understood. It is indeed a rather ineffective way to generate ATP and results in a release of high amount of lactate. The cell response to the tumor characteristic low O2 condition, called hypoxia, enables them not only to survive but also to resist most cancer therapies. Improvement of current therapies would thus benefit from a better understanding of cells response to hypoxia. In that regard, it is worth noting that most experimental setups designed to test putative therapeutics are performed under atmospheric (21%) O2 condition. This high condition neither reflects the tumor condition nor that of a tissue; it could even be considered as hyperoxia. In order to establish improved experimental conditions to study GBM cells, we investigated the response of 3 human GBM cell lines to hypoxic (1% O2), GBM in situ normoxic (3% O2) and atmospheric (21% O2) environments. Spheroids of tumor cells were generated, embedded in a collagen type I matrix and cultured for 7 days. Proliferation and invasion profiles, lactate release and activity of matrix metalloproteinases were analyzed and compared between conditions. Further O2 conditions are under investigation. This study shall help to define individual thresholds of hypoxia/in situ normoxia for each cancer cell line, a prerequisite to investigate and better characterize hypoxia-dependent metabolism in cancer cells.

INHIBITION OF APLN-APLNR SIGNALLING BLOCKS TUMOUR ANGIOGENESIS AND ATTENUATES INVASIVE GLIOBLASTOMA GROWTH

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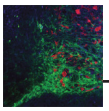
Glioblastoma multiforme (GBM) present with an abundant tumour neo-vasculature and a high degree of tumour cell infiltration into the parenchyma. Vascular endothelial growth factor-A (VEGF-A)



often resulted in accelerated GBM cell invasion. In a serial implantation model recapitulating the angiogenic switch of human GBM we found that the expression levels of the proangiogenic receptor APLNR and its cognate ligand APLN strongly increase with the onset of tumour-vascularisation. Screening a set of murine and human GBM stem cell (GSC) cultures of different genetic subtypes we consistently detected APLN/APLNR expression. Knockdown of APLN (AKD) in these GSCs and subsequent orthotopic implantation in mice massively reduced the GBM vascularisation, as compared to tumours generated by control GSCs. Injection of AKD-GSCs into APLN-knockout mice further normalised the intratumoural vascular network to a level otherwise observed in the tumour-free brain. Infusion of the APLNR-agonist apelin-13 restored angiogenesis in APLN-deficient tumours. In stereotactic biopsies of GBM patients we found an upregulation of APLNR in the tumour infiltrative zone (lacking aberrant vascularisation), which points towards an additional role for APLNR in tumor cells. In a series of cell culture and in vivo experiments we observed that autocrine APLN signalling promotes GSC-invasion. Importantly, application of an APLNR antagonist was able to block both tumour angiogenesis and GBM cell invasion in vivo. In summary, we show that APLN-APLNR signalling induces GBM neo-vascularization as well as GSC invasion and that both pathological features are blunted by APLNR-blockade. We propose APLNR inhibition as a new strategy for combined anti-angiogenic and anti-invasive GBM treatment, which may provide therapeutic benefits for a broad range of GBM subsets.

CD47-SIRPA BLOCKADE INDUCES A MICROGLIAL PHENOTYPIC SHIFT AND PROMOTES ACTIVE GLIOBLASTOMA PHAGOCYTOSIS IN VIVO.

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Modulation of tumor-associated macrophages and microglia (TAMs) in glioblastoma (GBM) presents as a promising anti-tumor strategy. However, little is known about the phenotypic organization of the TAM-pool and the role of GBM subtypes in shaping their specific immunologic environment. Therefore, we sought to model the composition of microglia and peripheral macrophages within the GBM-TAM-pool. Further, we aimed at assessing the response of the TAM pool to an important macrophage-modulating therapy, CD47-Sirp blockade. Using orthotopically xenografted, immunodeficient mice and syngeneic mouse models with genetically color-coded macrophages (Ccr2RFP) and microglia (Cx3cr1GFP), we found the TAM composition to be dependent on the rate of tumor growth. While microglia dominated TAMs in slow growing tumors, high passaged cell lines induced a mixed pool predominantly composed of microglia. Blockade of the "don't eat me" signal CD47 by anti-CD47 antibodies prompted macrophage and microglial-induced phagocytosis in vivo and lead to a marked microglial morphology change assessed by intracranial in vivo imaging. The therapeutic efficacy of anti-CD47 treatment was preserved in case of Ccr2-disruption and deficient macrophage recruitment to the brain. Anti-CD47 induced microglial phagocytosis alone was able to reduce tumor burden. Under anti-CD47 treatment, macrophages changed their transcriptional profile towards a more pro-inflammatory and M1-polarized signature whereas microglia upregulated phagocytic effector genes but was devoid of an inflammatory response. These results emphasize the importance of both resident microglia and invading macrophages in GBM biology. Moreover, CD47-Sirp alpha disruption caused an important phenotypic and functional status change of resident microglia, which will have implications for central nervous system (CNS) immunotherapies in the future.

GENERATION, CHARACTERISATION AND TREATMENT OF NOVEL MURINE MODELS FOR HUMAN PEDIATRIC GLIOMA

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In the last few years, it has become increasingly more evident that not every tumor within a tumor class is the same, and should therefore be treated differently according to the individual genetic alterations it harbors. Within the ICGC PedBrain Tumor Project, we analyzed almost 150 samples of low-grade and high-grade pediatric gliomas with whole-genome DNA and RNA sequencing, from which we were able to identify several genetic alterations likely responsible for tumor growth. Low-grade gliomas typically harbored alterations at different levels in the MAPK signaling pathway, including point mutations, fusions and duplications. Novel fusion events involved the NTRK2, FGFR1 and MYB oncogenes. Tumors with the latter alteration can also be defined as a distinct subgroup by DNA methylation profiling. In order to identify possible targeted therapy options for a variety of different pediatric low-grade gliomas, mouse models recapitulating the human disease are required on which specialized inhibitors can be tested. This project aims at generating, characterizing and finally treating these new individualized mouse models with specific gene or pathway inhibitors. Initial results with BRAF V600E and and NTRK fusion construct demonstrated the suitability of our somatic gene transfer method for generating new orthotopic models of human brain tumors, and we are now expanding this approach to additional candidate genes. In addition to the basic biological understanding of tumorigenic mechanisms that can be elucidated using these models, the results of this study will also have a translational aspect, which we hope will directly benefit patients in the clinic.

References: Jones et al. (2013), Nat Genet; Zhang et al. (2013), Nat Genet; Bender et al. (2016), Nat Med ; Gronych et al. (2011), JCI; Friedmann-Morvinski et al. (2012), Science

NEUTRALIZING GP130 INTERFERES WITH ENDOTHELIAL-MEDIATED EFFECTS ON GLIOBLASTOMA STEM LIKE CELLS

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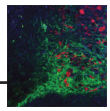
Glioblastoma (GBM) is the most common and aggressive primary brain tumor and is one of the most lethal forms of human cancer. Current therapeutic approaches remain largely ineffective and GBM almost invariably recur, resulting in a median survival of less than 15 months. A great deal of attention has been given to glioblastoma stem-like cells (GSCs), because of their key role in operating as the initiating and chemoresistant propagating tumor population. GSCs are found in the vicinity of brain endothelial cells, suggesting that functional interactions take place in the tumor vascular niche (Calabrese et al., Cancer Cell 2007). Through the development of an original in vitro co-culture model of human brain endothelial cells with patient-derived GSCs, we demonstrated that endothelial-secreted factors positively control the expansion and survival of GSCs (Galan-Moya et al, EMBO Rep 2011, Galan-Moya et al PLoS One 2014). The function of the factors emanating from endothelial cells in maintaining GSCs fate, plasticity, and survival urges to define the nature of these signals. We report that anti-gp130 neutralizing antibodies halt patient-derived GSCs expansion cultured in the presence of the endothelial cell-conditioned media. We found treatment with these blocking antibodies reduces GSC self-renewal,



as monitored by limiting-dilution assays and tumorsphere formation (Jacobs et al, Cell death and differentiation 2016). A recent article by Shi et al. (Cell death and differentiation 2016) demonstrates the importance of gp130 in maintaining stemness of GSCs and suggests targeting GP130 complexes for therapy. However, we provide clear evidence that neither the stem identity, nor cell survival is affected in defined complete medium. Altogether, our work brings a different perspective to the previous study on the function of gp130 signaling in GSCs, and will certainly foster additional studies by expanding upon published data and clarifying the therapeutic potential of pharmacological targeting of gp130 signaling in GBM.

NVIRONMENTAL FGF2 INDUCES ZEB1 EXPRESSION IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and malignant brain cancer in adults. Its aggressive growth and resilience to therapies is due to a high heterogeneity as well as the persistence of cancer stem-like cells with the potential for clonal expansion. We have previously demonstrated that ZEB1 (Zinc finger E-box Binding Homeobox 1) is a key regulator of stemness in glioblastoma, but it remains unclear how ZEB1 expression is controlled in these tumours. Searching for extrinsic inducers of ZEB1 in the glioblastoma microenvironment, we performed cytokine array analysis of patient specimens grouped according to ZEB1 expression. These arrays showed consistently high levels of Fibroblast Growth Factor 2 (FGF2). Previous studies have shown that FGF2 signalling stimulates GBM growth. FGF2 is highly prevalent in the CNS and is an essential mitogen for the maintenance of neural precursor cells and brain cancer stem cells, but the underlying molecular mechanisms are incompletely understood. Here, we hypothesise that FGF2 contributes to glioblastoma stemness and growth through ZEB1. We demonstrate that FGF2 increases sphere formation and ZEB1 expression in primary glioblastoma lines, and that this is mediated through specific FGF receptors (FGFRs). Moreover, we find that ZEB1 reciprocally affects expression and alternative splicing of FGFRs, indicating the existence of a feedback loop enabling ZEB1-expressing glioblastoma cells to thrive in the tumour microenvironment. Thus, this study proposes a link between FGF2 signalling and ZEB1 expression in glioblastoma, indicating a possibility of isolating cancer stem-like cells by FGFR profiles that may yield new targets for therapy.

CD44 INTERACTS WITH HIF-2A TO MODULATE THE HYPOXIC PHENOTYPE OF PERINECROTIC AND PERIVASCULAR GLIOMA CELLS

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Hypoxia-inducible factors enhance glioma stemness, and glioma stem cells have an amplified hypoxic response despite residing within a perivascular niche. Still, little is known about differential HIF regulation in stem vs. bulk glioma cells. We show that the intracellular domain of stem cell marker CD44 is released at hypoxia, binds HIF-2α (but not HIF-1α), enhances HIF target gene activation, and is required for hypoxia-induced stemness in glioma. In a glioma mouse model, CD44 was restricted to hypoxic and perivascular tumor regions, and in human glioma, a hypoxia signature correlated with CD44. CD44ICD was sufficient to induce hypoxic signaling at perivascular oxygen tensions, and blocking CD44 cleavage decreased HIF-2α stabilization in CD44-expressing cells. Our data indicate that the stem cell marker CD44 modulates the hypoxic response of glioma cells, and that the pseudo-hypoxic phenotype of stem-like glioma cells is achieved by stabilization of HIF-2α through interaction with CD44, independently of oxygen.

NEWLY IDENTIFIED PERICYTE-PROGENITOR CELLS PROMOTE GBM-ANGIOGENESIS

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Pericytes control angiogenesis as well as blood-brain barrier integrity in the developing CNS and in gliomas. Pericytes were postulated to derive from mitotically active, mature pericytes, which can be identified by the expression of markers including PDGFR-B, NG2, desmin or CD146. Using a newly established lineage-tracing mouse-model we show that mature pericytes largely originate from marker-negative cells. In this model stable fluorescence-reporter activity was obtained after tamoxifen (TAM) inducible genetic recombination controlled by a modified nestin-promoter element (NES-creER2). To study neo-angiogenesis in the adult brain we orthotopically inoculated syngeneic glioma cells and observed angiogenesis as well as pericyte-expansion over time. A smaller number of fluorescent cells was detected at early tumourigenesis (7 days after tumour implantation; P7), these had an amoeboid-like morphology and were located distant from the vasculature. At P14 and P21, when larger and strongly angiogenic tumours had formed, the progeny of traced cells had strongly expanded, was closely associated with the endothelia and exhibited an elongated cell-shape. Strikingly, the vast majority of recombined cells (78%) was negative for all pericyte-markers at P7, but acquired pericyte-markers (81%) at P21 – suggesting that mature pericytes derive from undifferentiated progenitors. These pericyte progenitors are not restricted to pathological angiogenesis, but were also observed in the developing CNS. Lineage-tracing experiments in bone-marrow chimeric glioma models indicated that pericyte progenitors are endogenous to the brain. Targeting pericyte progenitors in lineage ablation studies efficiently reduced the number of recombined cells, diminished the tumoural vascular network and decreased the tumour-burden by 50%. Our data indicate that newly generated pericytes origin from a previously unrecognised, brain-resident pericyte progenitor which can provide an excellent target for new anti-angiogenic treatments.

HUMAN MESENCHYMAL GLIOBLASTOMAS ARE CHARACTERIZED BY AN INCREASED IMMUNE CELL INFILTRATION COMPARED TO PRONEURAL AND CLASSICAL TUMORS

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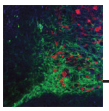

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Glioblastoma (GBM) is the most common and aggressive malignant primary brain tumor in adults, with a dismal median survival of 14.6 months following diagnosis. Recent research efforts have focused on identifying clinically relevant subgroups to allow for an improved understanding of pathogenetic mechanisms and patient stratification. Concurrently, the role of the cellular tumor microenvironment, especially tumor-associated macrophages and T cells, has received growing attention. On this basis, the present study seeks to assess differences in immune cell infiltration among distinct GBM subtypes. Patient-derived human GBM tissue samples were genetically characterized and assigned to the subtypes Proneural, Mesenchymal and Classical defined by NanoString nCounter Technology. Subsequently, automatic immunohistochemical staining for tumor-associated macrophages (IBA1+) and different T cell populations (CD3+, CD8+, FOXP3+) was carried out. Image quantification was performed with Fiji. Immune cell infiltration was significantly increased in the Mesenchymal subtype. In addition, all T cell subsets showed a positive correlation with tumor-associated macrophages. Based on these findings, a mathematical model was established, which was able to identify Mesenchymal GBM with a sensitivity of over 90% using a combination of those cell-specific markers. Given the increasing relevance of immunotherapeutic approaches in the treatment of GBM, this study provides a rationale for a potential clinical benefit as well as the feasibility of patient stratification based on molecular and cellular characteristics.

INFLUENCE OF LOW DOSE ORLISTAT ON GLIOBLASTOMAS' FATTY ACID METABOLISM

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With regard to possible clinical use we investigated the effects of chronic exposure to low dose Orlistat (27.8µM) on viability, autophagy, and genes participating in fatty acid metabolism in glioblastoma (GBM) cell cultures. Three primary human GBM cell cultures (G1145, G1273, G1319) were incubated with vehicle control or 27.8µM Orlistat for 96h. We analyzed cell viability by Prestoblue® and induction of autophagy by monodansylcadaverine staining. Furthermore we studied changes in expression levels of genes involved in fatty acid metabolism by qPCR: ATP-citrate-lyase (ACLY), acetyl-CoA-carboxylase 1 (ACACA), fatty acid synthase (FASN), and carnitine-palmitoyltransferase 1A (CPT1A). We revealed in all treated GBM-cultures a significant decrease of cell viability up to 79.0%, 96.6% and 253.2% after 96h (p=0.000) incubation with Orlistat with a positive significant correlation to time of exposure in G1319 (p=0.040, r=0.896). In G1145 and G1273 Orlistat increased autophagy significantly up to 15.8% respectively 16.0% after 48h (p=0.000). ACLY showed decreased (p=0.025), ACACA and FASN increased mRNA expression levels after 48h, and CPT1A decreased levels after 24h (p=0.004). Orlistat influences the fatty acid metabolism in investigated primary GBM-cultures even in low concentrations. The higher the growth rate of the analyzed cells, the more Orlistat affects cell viability negatively. To escape inhibition of Orlistat by binding FASN, its mRNA is upregulated. The functional inhibition results in malonyl-CoA accumulation and palmitate deprivation. This furthermore leads to adaptations on mRNA level of different genes but not in a constant manner. GBM cells may counter metabolic changes induced by Orlistat so inhibition of fatty acid metabolism should be performed in more than one way in future.

EUKARYOTIC INITIATION FACTORS MIGHT REPRESENT A NOVEL MARKER TO MONITOR THE EFFECTIVENESS OF GLIOMA THERAPY

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Objectives: Glioblastoma (GBM), the most malignant form of gliomas, is a particularly devastating neoplasm with a highly infiltrative nature. The outcome of patients with GBM is still very poor as current therapies combining surgical resection, combined radiation and chemotherapy can only marginally improve overall survival. Uncontrolled cell growth and malignant behavior are caused amongst others by altered protein synthesis. Translation initiation, regulated by eukaryotic initiation factors (eIFs), is one rate limiting step of protein synthesis. Few eIFs have been already shown to be altered during gliomagenesis. Thus, the aim of the study was to investigate expression patterns of the major eIF subunits upon treatment. Methods: GBM mouse xenografts models were treated with established therapeutic agents and screened for their effects on eIF expression and mTOR related proteins. eIF expression was evaluated upon administration of temozolomide, bevacizumab, everolimus, irinotecan, regorafenib, sorafenib and salinomycin compared to phosphate buffered saline. Results: Temozolomide, the most effective anti-proliferative agent in glioma treatment, totally down-regulated the expression of almost all eIF subunits. Interestingly, in case of temozolomide-resistance eIF expression remained constant or even increased upon treatment. Besides the alkylating agent temozolomide, decreased eIF levels were also detected after treatment with the tyrosine kinase inhibitor regorafenib. Combination treatments with temozolomide and other therapeutic agents did not reveal any additional reduction of eIF expression compared to mono-therapies. Conclusion: eIFs are not only up-regulated during gliomagenesis, but also seem to reflect the effectiveness of glioma therapies. Thus, eIFs might be used as novel marker to monitor the therapeutic effectiveness and may furthermore represent novel therapeutic targets in glioma therapy.

CMV INFECTION STIMULATES TUMOR CELL – PERICYTE CROSSTALK TO FACILITATE ANGIOGENESIS AND MIGRATION IN GLIOBLASTOMA

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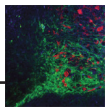
CMV has long been associated with various tumours and its interrelation with glioblastoma (GBM) was first described in 2002. Since then, several groups demonstrated that human malignant gliomas are universally infected with CMV and express gene products that regulate key signalling pathways in GBM pathogenesis. However, there is a lack of strong mechanistic data and other factors that mean this area is still fairly controversial. Here we present novel



regulatory mechanisms of CMV, that lead to changes in the cross talk between GBM cells and vascular pericytes, increasing angiogenesis and tumor cell migration. Using RNAseq, we analysed human brain vascular pericytes (HBVP) and GBM cells to identify differentially regulated genes after CMV infection. Our analysis revealed up-regulation of oncogenic tyrosine kinase receptors, and their ligands. We show that co-culturing of human brain microvascular endothelial cells (HBMEC) and infected GBM cells lead to the establishment of larger (160%, p=<.0001) and more complex (number of junctions 9.5 (-) vs. 21 (+), p<.0001) tube formation on Matrigel in vitro. Endothelial cell migration is likewise increased in the aortic ring assay and most likely driven by IL-6 and PDGF ligand secretion. Furthermore, HBVP migrated towards CMV infected GBM cells (increase 1.48-fold (p=0.009)) in a PDGF ligand dependent manner. This migration can be interrupted using neutralizing anti-PDGF antibodies in transwell migration assays. CMV infection and treatment with conditioned media derived media from infected HBVP, lead to upregulation of the pro-invasive surface receptor c-MET, an indicator of poor prognosis in GBM, in human and murine tumour cells. In- contrast, infected HBVP upregulate secretion of various ligands including the inflammatory cytokine IL-6. These changes of the secretome proved capable of activating tyrosine kinase receptor signalling. Transferring our findings to an in-vivo model, we report for the first time the generation of a latent MCMV mouse model using C57Bl/6 mice and GL261fluc allografts to mimic clinical circumstances of GBM patients. Tumour growth and vascularisation were significantly increased in these CMV infected mice and led to shortened survival times. In our model, tumours showed significantly higher numbers of infiltrating pericytes and tumour vessels. In summary, our data indicates that CMV infection leads to distinct changes in the secretome of GBM tumour- and associated perivascular cells. These changes increase the angiogenic potential and invasiveness in GBMs. We have been able to demonstrate that these changes are valid in vitro and in vivo, as well as apply to murine and human cell lines. Thus, CMV influences the causes of GBM progression by increasing angiogenesis and attracting pericytes to stabilize the tumour supplying vessels.

SYSTEMATIC XENOTRANSPLANTATION OF GLIOMA STEM CELL LINES FOR PHARMACOLOGICAL STUDIES ACROSS A WIDE RANGE OF TUMOR BEHAVIORS

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Background: Most xenograft models do not fully recapitulate the molecular heterogeneity or the diffusive growth pattern of glioblastoma tumors. In a multi-disciplinary project, we have established and characterized a large panel of patient derived glioma stem cell (GSC) lines with the goal of developing a platform to model patient-derived glioma stem cells in mice, linked to molecular and functional data. Methods: A total of 32 individual patient-derived GSC cultures have been engineered to constitutively express GFP and the firefly luciferase gene (luc2) and injected into mouse brain to allow in vivo monitoring of tumor growth. Bioluminescence signals and histopathological scoring of derived brain sections were applied to score tumor initiation in the xenotransplanted tumors. Finally, to score tumors for invasion, we invented a protocol by which automated image analysis is applied to scans of mouse brain sections stained for tumor cells. Results: Tumors displaying a wide range of histopathological features and biological behaviors recapitulating high grade astrocytoma were confirmed in mice injected with 15/29 GSC lines (52%). Glial lineage markers, such as Sox2, GFAP, and Olig2, were expressed both in patient tumors and in patient derived xenografts. Tumor initiating GSC lines had superior sphere forming capacity (p = 0.0018, Mann-Whitney). Further, we show that in vivo tumor aggressivity is correlated with stemness (p = 0.08, Pearson r = 0.71). Conclusion: Our collection of GFP-luciferase labeled glioma stem cell lines enable non-invasive quantification of disease progression and therapeutic response providing a valuable tool for conducting preclinical studies of drugs targeting predicted vulnerabilities in glioma and biomarker discovery.

EFFECT ON GLIOMA CELL VIABILITY BY ENHANCED PROTEIN LOAD STRESS FOLLOWING PROTEASOME INHIBITION

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Background: Bortezomib, a reversible proteasome inhibitor, is effective against many cancer cell types and is used clinically in the treatment of multiple myeloma and mantle cell lymphoma. Through a systematic screening of drug response in the well characterized and heterogeneous collection of glioma stem cell (GSC) lines, we identified a bimodal response to proteasome inhibitors, ranging between tolerance to the drug in some cell lines, to extreme sensitivity in others. Methods: In this study, we further explore the functional response to Bortezomib treatment in sensitive and tolerant GSC lines with particular emphasis on protein metabolism and cellular stress response. Results: Our functional analysis show that sensitive glioma stem cell lines have an increased proteasomal flux and accumulate polyubiquitylated proteins in response to treatment. The failure to clear misfolded proteins in sensitive cells lead to aggresome formation and autophagic clearance. The ratio of reduced to oxidized glutathione is lower and the rate of apoptosis is higher in sensitive cells, supporting a mechanistic response involving an overload of misfolded proteins resulting in oxidative stress, DNA damage, induction of p21 and apoptosis. Conclusions: The cellular stress response observed in sensitive glioma cells following proteasome inhibitor treatment suggest that combination therapy involving drugs that interfere with DNA damage response or the cellular redox potential could enhance the effect of proteasome inhibitors in drug tolerant glioma stem cells. Further characterization of the underlying molecular phenotype that explains why tolerant cells are responding less to such stress is ongoing.

RNA SEQUENCING OF GL261 CELLS AND TUMORS AND TCGA DATA POINT TO CD74 AS A MAJOR PLAYER IN TUMORIGENIC GLIOMA MICROENVIRONMENT

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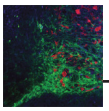
The most frequently used murine glioblastoma (GBM) model is GL261. Despite its frequent usage, there is little information on the biological and genetic features underlying vivo development of GL261 gliomas. The aim of our study was to define mutations and gene expression changes in GL261 neurospheres and tumors in the syngeneic hosts, C57BL/6N mice. GL261 cells were cultured as neurospheres in a stem cell growth medium. C57BL/6N mice received intracranial injections of GL261 cells into the caudatum. After injection, individual symptomatic mice were euthanized and tumors excised. Exome and RNA sequencing of GL261 cell line, tumor derived and normal brain was performed in collaboration with BGI TECH SOLUTIONS using Illumina HiSeq technology. Exome in normal brain, GL261 and in derived tumor contained 600 non synonymous somatic mutations. Of these 298 were filtered as uncommon dbSNP



and 108 were expressed: 92 missense, 6 stop gained and 3 site splicing. We found in cell line and tumor 2 mutations on KRAS gene and on TP53 gene described by Szatmari et al. (2006). We also identified 535 differentially expressed genes (fold change>1): 381 upregulated and 154 down-regulated in tumors vs cells. One of the most genes highly upregulated was CD74, which encodes a protein associated with major histocompatibility complex class II. Notably TCGA data showed that in grade II-III gliomas the expression of CD74 is inversely correlated with the survival of patients ($p<0.0001$). These data point to CD74 as a major protumorigenic driver in glioma microenvironment and suggest that more work should be developed to unveil its potential as therapeutic target.

DIFFERENT PROMOTER ELEMENTS OF THE NESTIN GENE PROVIDE LINEAGE TRACING MODELS FOR NEURAL PRECURSORS OR PERICYTE PROGENITORS

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Transgenic mouse models are instrumental to trace stem cell progeny and cell-lineage relationships. Here we used two tamoxifen-inducible lineage-tracing mouse models with different nestin-promoter sequences (Neslong-CreER2 and Nesshort-CreER2) in order to study the contribution of parenchymal cells to experimental gliomas of the adult brain (postnatal day 90; P90). At this stage adult neurogenesis has largely declined and reporter activity in recombined cells (red fluorescent protein; RFP) was low in peritumoural areas and almost absent within tumours in the Nesshort-CreER2 model. Cells traced towards the peritumoural region expressed GFAP, in line with a neural precursor cell-origin. The cell-numbers and differentiation states of RFP-positive cells in the Neslong-CreER2 glioma model were strikingly different. Here, large numbers of RFP+ cells could be observed within in the tumour mass 14 days after orthotopic glioma cell injection, while much less recombined cells were visible in the tumour perimeter. High numbers of traced cells were found throughout aging (at P90 and P360) in this glioma model and no glial-, neuronal-, endothelial- or myeloid-lineage commitment was detected. Strikingly, traced cells in the Neslong-CreER2 glioma model acquired a pericyte identity over the time course of gliomagenesis and expressed markers like desmin, PDGFR-B, NG2 or CD146. Thymidine-analogue labeling paradigms indicated that a substantial fraction of traced cells was continuously in the cell cycle. Furthermore, we investigated if the Neslong-CreER2 model could also indicate the formation of pericytes in physiological angiogenesis. Hence, we investigated the vascularization of the developing retina and again found that RFP+ cells acquired a pericyte phenotype. All in all, we report the Neslong-CreER2 model as a valuable tool to decipher the lineage relationships of vascular mural cells and to trace the point of origin of newly generated pericytes.

TARGETING THE HEDGEHOG-SIGNALING PATHWAY IN GLIOMA STEM-LIKE CANCER CELLS

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Gliomas are the most common brain tumors in adults. They are characterized based on histological and genetical markers with the most aggressive type being the glioblastoma (WHO grade IV). Due to their highly invasive growth and infiltration of the surrounding tissue is the median survival only slightly above 15 months with a 5-year survival rate below 5% using the currently available standard therapy. Thus new and effective treatment modalities are of significant importance. One possible mechanism that is currently discussed in being responsible for tumor recurrence is the presence of a subpopulation of tumor cells with stem-like properties. The Hedgehog (Hh) signaling pathway was initially described as a developmentally essential signaling pathway that is mainly silenced in adults. During the last years it was shown, however, that the Hh signaling pathway is causative and also implicated in a variety of human cancers, including glioblastoma. One key aspect of Hh signaling is the regulation and maintenance of stem cells in its physiological role as well when deregulated. Thus it was shown that Hh signaling is active and required for the maintenance of cancer stem-like cells in various cancers as was shown for glioblastoma. Here we provide evidence that the Hh pathway is a reasonable target in glioblastoma stem like cell lines. We can show that the inhibition of the GLI transcription factor strongly inhibits proliferation and induces cell death. Since glioblastomas are known for their vast intrinsic heterogeneity we decided to target additional pathways. Of the compounds tested we found that the pan-BCL2-inhibitor AT-101 ((-)-gossypol) synergistically increases the antitumoral responses of Hh inhibition. Thus we conclude that this combination represents a novel, promising treatment option for glioblastomas.

THE POTENTIAL OF CRL4DCAF1 AND KSR1 AS THERAPEUTIC TARGETS IN MERLIN-DEFICIENT TUMOURS

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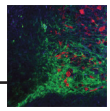
BACKGROUND: Merlin-deficient meningiomas are caused by mutations in the Neurofibromin 2 gene and occur in approximately 60% of sporadic meningiomas. Merlin loss is commonly associated with the genetic condition Neurofibromatosis type 2, leading to the development of multiple low grade tumours including schwannoma, meningioma and ependymoma. Currently, the only treatment for these tumours is (radio)surgery and therefore identification of novel drug targets is vital. Previous studies have shown that Kinase suppressor of Ras 1 (KSR1) is a potential therapeutic target in schwannoma and that the E3 ubiquitin ligase, CRL4DCAF1, binds to KSR1. The aim of this project is to investigate the interaction between CRL4DCAF1 and KSR1 to determine if targeting this protein complex in schwannoma and meningioma holds therapeutic value. METHODS: HEK293T cells were transfected with KSR1 constructs and interactions with CRL4DCAF1 were investigated by immunoprecipitation. Immunohistochemistry, cell fractionation and Western blot were used to analyse DCAF1 and KSR1 expression and localization in primary human tumour cells. A shRNA construct was used to knock down DCAF1. The inhibitors, MLN3651 and APS_2_79, were used to inhibit DCAF1 and KSR1, respectively. RESULTS: KSR1 interacts via the N-term with DCAF1 and is ubiquitinated at the C-term in the NF+/+ model, which may be DCAF1 dependent. DCAF1 knockdown led to a reduction of proliferation in schwannoma and meningioma. MLN3651 reduces cell viability and proliferation of meningioma as well as inhibiting the hippo pathway. However, both DCAF1 knockdown and MLN3651 treatment in meningioma led to an increase in pERK. Combination of shDCAF1 and APS_2_79 reduced pERK and proliferation in both BenMen-1 (a benign meningioma cell line) and primary meningioma. Therefore, targeting both DCAF1 and KSR1 represents an attractive novel therapeutic strategy in meningioma.

UNVEILING THE CELLULAR AND MOLECULAR CHANGES OF THE MICROENVIRONMENT DURING BRAIN TUMOR DEVELOPMENT

Prerna Magod1, Lilach Agemy2, Liat Rousso-Noori1 and Dinorah Friedmann-Morvinski1,3



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Glioblastoma (GBM) is an aggressive, highly invasive primary brain tumor with near total fatality. GBM remains a challenge for prognosis despite intensive therapies including radiation, chemotherapy and surgery. Our lab uses a Cre-inducible lentiviral vector-based mouse model of GBM that faithfully recapitulates the pathophysiology of the human disease. Transduction by oncogenic lentiviral-vectors of neural stem cells, astrocytes or even mature neurons in the brain of mice can give rise to gliomas. All the tumors, irrespective of the initiating cell population share a common stem-like cancer cell population that can originate from reprogramming or de-differentiation of mature transformed cells. We believe that the tumor microenvironment (TME) may contribute to the process of tumor reprogramming. Using our mouse model of GBM we set to characterize the composition of the brain TME periodically by flow cytometry and immunohistochemistry during tumor progression. Preliminary results showed differences in both the innate and adaptive immune populations of the brain TME when compared to healthy brain tissue. Surprisingly, we also observed changes in these immune cell populations in the spleen of tumor-bearing mice compared to healthy mice. To further characterize the tumor-stroma interactions in GBM we plan to study the longitudinal transcriptomic and epigenomic changes of the TME along tumor progression. Our findings will facilitate the development of novel strategies aiming at reprogramming the TME as an additional approach to attack cancer.

OPTICAL BARCODING: A NEW TECHNIQUE TO ANALYZE TUMOR HETEROGENEITY

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Most of the genomic and epigenetic alterations in glioblastoma (GBM) which have been described so far, highlight the highly heterogeneous and complex genomic landscape of GBM. To develop more successful therapies we need to understand the heterogeneity of GBM at the single cell level in order to eliminate the emergence of drug resistant clones. We generated a new in vivo and in vitro model that maintains the cellular heterogeneity of the original tumor by establishing GBM-patient derived cell lines (PDCL) and mouse xenograft (PDX) from tissue resections. To be able to follow the growth progression of multiple clones in vivo we took advantage of an optical barcoding system to track and quantify clonal evolution. Using a combination of 6 different color-coded lentiviral vectors we isolated single cells out of GBM-PDCL and tagged them with 21 different color combinations. Using this innovative technique, we tracked the fate of individual clones and analyzed their growth dynamics in different microenvironments. We discovered that fast growing clones in vitro are not necessarily the ones responsible for tumor growth. Moreover, we discovered that some clones are more resistant to radiotherapy and standard of care treatment than others, opening the possibility to identify and analyze specific clonal subpopulations in the tumor that are resistant to treatment. This, in turn, would help to predict drug treatment efficiency and potential resistance.

IMMUNOESCAPE DURING GLIOMA PROGRESSION

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Glioma is a primary brain tumor whose grade IV, glioblastoma, is the most frequent and incurable form. To study the mechanisms involved in glioma progression, we generated a mouse model of glioma by overexpressing the PDGF-B oncogene in embryonic neural progenitor cells in vivo. We demonstrated that all the animals develop tumors that initially show a low-grade phenotype and do not form new tumors when transplanted in syngeneic adult animals. However, these tumors invariably undergo a malignant progression towards a high-grade phenotype with tumor-propagating potential in vivo. A microarray analysis on these tumors revealed a strong downregulation of immune-stimulatory genes during malignant progression. Moreover, immunistochemical and citofluorimetric assays showed that high-grade tumors have a lower immune infiltration of CD45 and CD8 positive cells. These data suggest that an important step during glioma progression is the acquisition of the ability to hide from the immune system thus preventing an immune response. More strikingly, we noticed that immunodeficient mice represent a permissive background for low-grade gliomas transplantation and progression. Cells obtained from low-grade tumors generate secondary gliomas in Nod/Scid mice and, surprisingly, acquire the ability to graft in immunocompetent mice. All these data strongly suggest a cross-talk between glioma and immune cells that regulate glioma formation and progression.

ROLE OF A BETA INTEGRIN IN STEMNESS MAINTENANCE AND RADIORESISTANCE OF GLIOBLASTOMA-INITIATING CELLS

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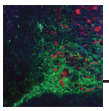
Glioblastomas (GB) are malignant brain tumors with dismal prognosis despite standard treatment which includes maximal surgical resection followed by fractionated radiotherapy with concomitant and adjuvant chemotherapy (Temozolomide). This severe outcome could partly be explained by the presence into the tumor of Glioblastoma-Initiating Cells (GIC), characterized by their ability to self-renew, their higher expression of specific GIC markers, their pluripotent aptitude to differentiate (neurons, astrocytes or oligodendrocytes), and their high tumorigenic potential. In addition, GIC are particularly chemo-radioresistant and involved in tumor recurrence. So, current research focuses on developing potential GIC-targeted therapies in order to improve GB treatment. Regarding current literature but also transcriptomic results obtained in our lab, a specific Beta integrin emerged as a potential selective target in GIC. We then hypothesized that this integrin could be involved in stemness maintenance but also radioresistance in GIC. We first demonstrated, with several primocultures from patients, that this Beta integrin is overexpressed in GIC in comparison to their differentiated progeny. Moreover, this integrin could be associated with characteristics and features unique to these cells, including self-renewal ability, viability, stemness status and radioresistance. Indeed, the selective inhibition of this Beta integrin in GIC by shRNA resulted in a decreased neurosphere formation associated with an increase of differentiation patterns and cell death, this one being potentiated after irradiation. These results could eventually allow to identify this Beta integrin as a new membrane marker of GIC but also to evaluate its targeting potential as a new therapeutic radiosensitizing strategy in these quite aggressive and invasive brain tumors.

LABEL-FREE MULTIPHOTON IMAGING FOR RECOGNITION OF HUMAN BRAIN TUMOR BORDERS BY TEXTURE ANALYSIS

Georg Mark[1], Ortrud Uckermann[1], Roberta Galli[2], Matthias Meinhardt[3], Gerald Steiner[2], Gabriele Schackert[1], Matthias Kirsch[1]



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Multiphoton imaging allows the assessment of morphochemical information at subcellular level without any labeling. The reliable intraoperative recognition of brain tumor borders and diagnosis of the tumor is a challenge in neurosurgery. Therefore, we used label-free multiphoton microscopy in conjunction with texture analysis to establish an observer-independent automated recognition of tumor borders. Cryosections of human glioblastoma, astrocytoma, oligodendroglioma and non tumor brain tissue were analyzed by CARS (coherent anti-Stokes Raman scattering), TPEF (endogenous two photon excited fluorescence) and SHG (second harmonic generation). Gray values of each modality were normalized in the dynamic range (min to max) and first order parameter (mean, standard deviation, kurtosis, skewness, entropy) and second order parameters (contrast, correlation, energy, homogeneity) were calculated using using Matlab. Images of white matter were dominated by axons (CARS), gray matter displayed cell bodies with fluorescent inclusions (TPEF) while glioblastoma had a homogenous appearance. These differences translated into tumor-specific texture parameters that allowed tissue classification with an accuracy of > 90% by linear discriminant analysis. Furthermore, supervised classification allowed the recognition of the tumor border if present in the sample. Combined CARS-TPEF-SHG images are sufficient to extract tissue characteristics using automated texture analysis. Therefore, observer-independent, reliable brain tumor classification and tumor border identification can be obtained. Based on this objective analysis, intraoperative multiphoton images could be classified in real-time to provide this information immediately to the surgeon.

CXCR4 – A NEW PLAYER IN VESTIBULAR SCHWANNOMA PATHOGENESIS

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CXCR4, a member of the chemokine receptor subfamily, plays a significant role in homing and recruiting progenitor and immune cells, especially during embryogenesis. It is also expressed in different solid cancers and increases tumor cell survival, growth, invasiveness and metastasis. To our knowledge, no data on CXCR4 expression in vestibular schwannoma (VS) have been published. We examined the CXCR4 expression in patients´ samples of VS of varying extensions with or without neurofibromatosis type 2 (NF2). CXCR4 mRNA and protein was extracted from frozen VS samples (n=60) of which 30 patients had NF2. The control group (n=10) consisted of 3 patients' peripheral sensory nerves and 6 normal vestibular nerves from autopsies. CXCR4 mRNA was measured by qPCR (2-ΔΔCT method) and protein by immunohistochemistry (IHC) and Western-blotting (WB). In a retrospective analysis the VS were categorized according to tumor extension and hearing loss by Hannover Classification. CXCR4 expression could be detected by all three methods. Its mRNA was 4.6x overexpressed in VS (NF2 patients 5x, sporadic VS 4x overexpression vs. control). A heterogeneous protein-expression profile was observed in IHC and WB, the latter correlating with the presence of multiple CXCR4 isoforms. In contrast to the tumor extension, there was a strong correlation of the CXCR4 expression with the level of functional impairment (r=0.98). Patients with a slight hearing loss had a 3.9x, patients with a medium one a 4.6x and patients with deafness a 5x overexpression of CXCR4 compared to the control group. Compared to normal vestibular nerves, CXCR4 was overexpressed on mRNA level independently to tumor growth but with a strong correlation of higher expression levels with enhanced functional impairment. Therefore, inhibition of CXCR4, as tested already in trials for breast cancer therapy, could also be a potential systemic approach for the treatment of VS.

PRIMARY GLIOBLASTOMA CELLS EXPLOIT APLN-APLNR SIGNALLING TO ATTRACT TUMOUR-ASSOCIATED MYELOID CELLS

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Glioblastomas (GBM) are devastating, treatment-refractory brain tumours. The rapid progression of GBM is fostered by GBM-induced immunosuppression and by tumour-associated myeloid cells (TAMs), which massively accumulate in GBM and support glioma expansion. Hence, signalling-pathways controlling the tumour tropism of myeloid cells or immune-regulation of TAMs constitute a potential therapeutic target for GBM. Using different GBM-mouse models, we found that the angiogenic factor APLN signals through its receptor APLNR to regulate neo-vascularization and tumour cell invasion, while APLNR-blockade could reduce overall GBM growth. When screening for APLNR co-regulated genes in the TCGA database they correlated best with lymphocyte-mediated immune responses. Therefore we asked if APLN-APLNR-signalling could play a role in GBM-immune cell interaction. We found that murine myeloid cells (primary microglia, BV-2 or J774) express APLNR and that the APLNR-agonistic peptide apelin-13 acted chemotactic. Orthotopic implantation in mice using human primary GBM or U87MG cells transduced with APLN knock-down vectors (AKD) or controls, demonstrated that attenuating APLN-expression decreased the number of TAMs and reduced tumour volume. Injection of AKD cells in APLN-deficient (aplnKO) mice further reduced TAM-density, as compared to APLN wild-type controls. In contrast, infusion of apelin-13 into AKD tumours that were implanted into aplnKO mice restored TAM accumulation. This was recapitulated in immunocompetent GBM models using transgenic murine GBM cells or the GI261 cell-line. Finally, a cell-culture model using different human AKD or control GBM-cultures indicated that migration of BV2 or J774 cells is controlled by APLN-release from GBM. Altogether, we show that APLN derived from both GBM-cells and tumour-vessels attracts myeloid cells. We suggest that blockade of APLN-APLNR signalling mediates broad anti-tumour effects by inhibiting angiogenesis and reducing the number of TAMs.

OVERCOMING THE BLOOD BRAIN BARRIER AND GENOMIC COMPLEXITY

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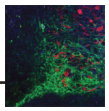
Introduction and Aims: Glioblastoma is the most malignant and common form of adult brain cancer. Patients only survive a median of 15 months after surgical resection and chemoradiotherapy. Despite knowledge of major involved pathways, treatment has not significantly improved in the last decade. This project seeks to highlight the potential use of a novel treatment method to overcome the blood brain barrier (BBB) and the importance of developing in silico models to analyze genomic complexity of the disease. Methods: Drug-loaded hydrogels were injected into ex vivo tumor tissue. After brief incubation periods, samples were fixed, cryopreserved, and



then sectioned. Fluorescent microscopy was used to examine the hydrogel-tissue interface. Early-stage, in silico glioma models were developed in BioModelAnalyzer (BMA) and F#. Results: Hyaluronic acid supramolecular hydrogels of cucurbit[8]uril have the potential to provide local drug delivery and avoid pitfalls experienced with Gliadel™ wafers. The hydrogels, with enzyme-cleavable cross-links for drug loading and sustained release, are malleable/mouldable, stimuli-responsive, and injectable. Fluorescent microscopy after injection revealed excellent tissue apposition and impressive drug diffusion. This proved true for two different cargos and incubation times. In vivo viability and efficacy studies will begin soon. A GBM signaling network is being built in BMA that allows model testing of observed perturbations and backwards analysis of a fixed phenotype. A 2D, off-lattice hybrid model that simulates physicospatial properties of early, avascular glioma spheroid development has been created in F#. Conclusions: The injectable hydrogel platform has the ability to provide sustained drug delivery directly into the tumor cavity following resection. BMA and F# models allow us to integrate and analyze complex genomic and physicospatial information to predict phenotypic consequences of targeted, combinatorial therapies in the context of a patient's genetic mutation profile.

MTOR PATHWAY IN GLIOBLASTOMA MULTIFORME: AN IN VITRO STUDY OF PP242, A NOVEL MTOR INHIBITOR

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mTOR is a kinase complex involved in cell growth, proliferation, survival, metabolism and mobility whose aberrant activation has been demonstrated in Glioblastoma multiforme (GBM), which makes it an interesting target for therapeutic approaches [1]. To date, attempts to block mTOR activity had disappointing clinical efficacy, as the mTOR inhibitor Rapamycin and analogs only target mTORC1 and since mTOR exists in two distinct complexes mTORC1 and mTORC2, that differ both in terms of regulation mechanisms and functions [2,3]. mTORC1 is inhibited by Rapamycin and acts as a downstream effector of the PTEN/PI3K/Akt pathway, linking growth factors, amino acids, ATP and O2 signals to protein translation, cell growth, proliferation and survival. Whereas, mTORC2 is insensible to Rapamycin and acts as an upstream activator of Akt via phosphorylation of serine 473 [3]. To analyze the contribution of mTORC2 to GBM biology, we studied the response of glioma cell lines of different malignancy degree to treatment with PP242, a novel mTORC1/2 inhibitor, compared to the response to Rapamycin and Wortmannin, a PI3K irreversible inhibitor. Results suggest that there is a variable response to PP242 and Rapamycin because of an unbalanced contribution of mTORC1 and mTORC2 in glioma cells of different malignancy degree, making the use of these drugs in clinical study problematic.

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AN ANTIBODY-GUIDED POLY-PROPYLENE-IMINE (PPI)-BASED POLYPLEX-SYSTEM FOR SIRNA-TREATMENT OF EGFRVIII-POSITIVE TUMORS

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Therapeutics based on small interfering RNAs (siRNAs) offer great potential to treat so far incurable diseases such as glioblastoma multiforme (GBM). However, the broad application of siRNAs is problematic. In order to overcome the obstacles, we have developed a single chain antibody fragment (scFv)-guided polyplex system for targeted delivery, based on transfection-disabled maltose-modified poly-propylene-imine siRNA carrier molecules. To achieve selective siRNA delivery into EGFRvIII-positive tumor cells, a specific anti-EGFRvIII single chain antibody (scFv(MR1.1)) was utilized and conjugated to polyplexes through a novel coupling strategy and evaluated in vitro and in tumor xenografts. The production of a scFv fused with a biotinylation acceptor peptide (P-BAP) sequence derived from Propionibacterium shermanii transcarboxylase in biotin ligase-expressing HEK293T cells leaded to functional mono-biotinylated scFv-P-BAPs. Polyplex formation was achieved by a sequential conjugation of scFv-P-BAP to NeutrAvidin and mono-biotinylated mal19-biotin at defined stoichiometry. Compared to polyplexes conjugated to an unspecific control scFv-P-BAP, the generated tumor-specific polyplexes were able to bind to EGFRvIII-positive target cells and to exclusively deliver siRNA by selective receptor-mediated endocytosis. Atomic force microscopy revealed stable polyplexes, with a mean diameter of 150 nm that circumvents fast renal excretion and therefore provided a further precondition for the specific accumulation of tumor-specific polyplexes in subcutaneous tumors of nude mice.

LONG-TERM PRODRUG ADMINISTRATION IMPROVES LENTIVIRAL VECTOR MEDIATED SUICIDE GENE THERAPY

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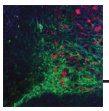
1K. G. Jebsen Brain Tumour Research Centre, Department of Biomedicine, University of Bergen, Norway; 2 Department of Pathology, Haukeland University Hospital, Norway; 3 Research Department Cell and Gene Therapy, Department of Stem Cell Transplantation, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, German; 4Norlux Neuro-Oncology Laboratory, Department of Oncology, Luxembourg Institute of Health, Luxembourg

We have previously shown that lentiviral vector mediated herpes simplex virus thymidine kinase (HSV-Tk)/ ganciclovir (GCV) therapy is a very promising therapeutic option for the treatment of glioblastoma (GBM). Although this therapy leads to complete remission of GBM in an orthotopic PDX model, recurrent tumors are observed which contain a fraction of Tk-GFP+ cells surviving 3-4 weeks of prodrug administration. We sorted Tk-GFP+ glioma cells from recurrent tumors and observed that the cells are less proliferative and retain sensitivity to GCV. Thus, we showed that short-term prodrug delivery- used in clinical gene therapy trials - fails to eliminate a fraction of glioma cells, which are slow proliferating; we hypothesized that a longer period of prodrug administration would provide an enhanced therapeutic effect. As long-term prodrug we used valganciclovir (valGCV), which is similar to GCV, but tailored for oral administration. Prolonged administration of valganciclovir (valGCV) resulted in a significant survival advantage compared to short-term (3 weeks) GCV application. Nonetheless, the majority of animals treated with valGCV also developed recurrent tumors. These tumors were more invasive compared to the primary tumors and showed significant upregulation of the epidermal growth factor receptor (EGFR). We are currently investigating signaling pathways upstream and downstream of EGFR. Our results warrant a treatment combination of Tk/ValGCV gene therapy with EGFR inhibitors, which we are currently investigating.



EFFECTS OF TIVOZANIB, A PAN-INHIBITOR OF VEGF RECEPTORS, ON GROWTH AND INVASIVE ABILITIES OF GLIOMA CELLS

Farima Moghaddaskho1, Majid Momeny1, Narges K. Gortany2, Hassan Yousefi3, Zahra Sabourinejad4, Ghazaleh Zarrinrad1, Haniyeh Eyvani1, Farinaz Barghi1, Azam Zaghal1, Ghazaleh Sankanian1, Fatemeh Esmaeili3, Zivar Alishahi3, Hoda T. Moghaddam1, Somayeh Vaezi-Joze1, Mahmoud Ghazi-Khansari2, Kamran Alimoghaddam1, Ardeshir Ghavamzadeh1, Seyed H. Ghaffari1

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Glioblastoma (GBM) remains one of the most fatal human malignancies due to its high angiogenic and infiltrative capacities. Even with optimal therapy including surgery, radiotherapy and temozolomide, it is essentially incurable. GBM is among the most neovascularised neoplasms and its malignant progression associates with striking neovascularisation, evidenced by vasoproliferation and endothelial cell hyperplasia. Targeting the pro-angiogenic pathways is therefore a promising anti-glioma strategy. Here we show that tivozanib, a pan-inhibitor of vascular endothelial growth factor (VEGF) receptors, inhibited proliferation of GBM cells through a G2/M cell cycle arrest via inhibition of polo-like kinase 1 (PLK1) signalling pathway and down-modulation of Aurora kinases A and B, cyclin B1 and CDC25C. Moreover, tivozanib decreased adhesive potential of these cells through reduction of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Tivozanib diminished GBM cell invasion through impairing the proteolytic cascade of cathepsin B/urokinase-type plasminogen activator (uPA)/matrix metalloproteinase-2 (MMP-2). Combination of tivozanib with EGFR small molecule inhibitor gefitinib synergistically increased sensitivity to gefitinib. Altogether, these findings suggest that VEGFR blockade by tivozanib has potential anti-glioma effects in vitro. Further in vivo studies are warranted to explore the anti-tumour activity of tivozanib in combinatorial approaches in GBM.

CAVEOLIN-1, A DRIVER OF INVASIVE PHENOTYPE IN IN-VITRO 3D-SPHEROID ASSAYS COMPRISED OF HIGH GRADE GBM CELLS ASSOCIATION WITH AN AKT-INHIBITED PHENOTYPE.

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1Cardiff University/ School of Pharmacy and Pharmaceutical Sciences; 2National Research Council of Rome/institute of Complex Systems; 3Cardiff University/European Cancer Stem Cell Research Institut; 4University of Portsmouth/Brain Tumour Research Centre of Excellence

INTRODUCTION: Glioblastoma multiforme (GBM) cells display a highly invasive phenotype, a hallmark which counters effective surgical and radiotherapy strategies. Caveolin-1 (Cav-1) is the main structural and functional component of caveolae. The impact of the expression of Cav-1 within a range of tumour and tumour-associated stromal cells is variable with both oncogenic and tumour suppressive roles reported which appear to be both disease-specific and context-dependent. Our hypothesis is that Cav-1 serves as promoter of invasion of GBM cells. MATHERIALS AND METHODS: To investigate our hypothesis we used a lentiviral shRNA approach to silence Cav-1 in three GBM cell lines (U87, UP007, UP029) derived from adult brain tumours. We employed an in-vitro 3D cell-sprouting invasion assay with GBM cell spheres embedded in Matrigel. Quantification of invasion was undertaken using a novel image analysis tool or 3D systems, INSIDIA (ImageJ Macro for High-throughput Spheroid Invasion Analysis). Parallel migration and invasion studies were performed using a Boyden Chamber approach, as well as cell-cell adhesion assays. Activation of signalling pathways in 2D and 3D cultures were performed by proteomic array and Western Blot analysis. RESULTS AND CONCLUSION: GBM cells expressing Cav-1 (Cav-1 +ve) displayed a higher invasive capacity compared cells where Cav-1 had been silenced Cav-1 –ve), the latter also showing increased cell-cell adhesion. A significant finding from the signalling analysis was an inverse association between Cav-1 silencing and activation of AKT evidenced by increased phosphorylation at both Ser473 and Thr308 sites. Ongoing studies are exploring this signalling axis and its relationship to the invasive phenotype.

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DECIPHERING LRIG1 TUMOR-SUPPRESSING SIGNALING IN GLIOMA

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Glioblastoma (GBM) is the most frequent and aggressive primary tumor of the human brain. Despite multimodal therapeutic strategies, tumor relapses systematically occur and are associated with very bad prognosis. LRIG1 (Leucine-rich Repeats and ImmunoGlobulin domains protein 1) was identified as an inhibitor of the epidermal growth factor receptor (EGFR) and was later confirmed as a tumor suppressor protein. We have shown previously that local delivery of soluble LRIG1 (sLRIG1) into the brain leads to a drastic reduction of GBM growth in mice. In this project, we aim to precisely depict the molecular sequence of events that is elicited by sLRIG1 and that leads to tumor blockade. We first confirmed sLRIG1 inhibitory effect on GBM cell line, showing that sLRIG1 overexpression leads to reduced proliferation, reduced invasion, and downregulation of EGFR. Interestingly, we also observed that sLRIG1 overexpression is associated with downregulation of other receptor tyrosine kinases (RTKs), and induces significant changes in gene expression. Later on, we validated these observations with a purified recombinant human sLRIG1 (rh-sLRIG1) protein that we used as a soluble treatment of GBM cell lines, showing that rh-sLRIG1 efficiently reduces EGFR expression, proliferation and invasion. We therefore confirmed that rh-sLRIG1 might constitute a novel therapeutic molecule for the inhibition of growth factor signaling in GBM, and that this project could pave the way for an unexplored therapeutic strategy against this dismal cancer.

THE HGCC FUNCTIONAL ATLAS UNCOVERS MODULAR NETWORKS OF DRUG SENSITIVITY IN GLIOBLASTOMA CELLS

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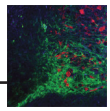
Background The treatment of glioblastoma is one of the outstanding challenges of cancer research. We have developed a library of annotated and validated cell lines derived from surgical samples of GBM patients, maintained under conditions to preserve stem cell characteristics. This collection, which we call the Human Glioblastoma Cell Culture (HGCC) resource, comprises 176 GC lines and an associated database containing high-resolution molecular data (hgcc.se). The HGCC lines are tumorigenic, harbor genomic lesions characteristic of GBMs, and represent all four transcriptional subty-



pes. Methods Leveraging this material as an opportunity to explore differences in drug response across well-annotated glioblastoma cell cultures, we studied the spectrum of sensitivities to drug-like compounds in 115 HGCC lines. Robust multivariate techniques were used to predict the vulnerability of 248 compounds from multiple layers of genomic data. Results. Our integrated analysis confirmed known drug sensitivities and identified new associations between compound sensitivity and molecular biomarkers. In all, four organizing principles emerge:
(i)Drug activity is highly modular in such that compounds are highly correlated based on chemical class and target.
(ii)Existing molecular subtypes are poor pedictors of drug modules.
(iii)As a more relevant stratification, we introduce a global model by which each drug module is determined by unique combinations of transcriptional, mutational and epigenomic factors.
(iv)The most variable module was characterized by an extreme and unexpected degree of variation in their response to proteasome inhibitors (PI).
In support of our findings, the new PI module was corroborated by a xenograft treatment study (n=41) that showed a significant difference in response between the two classes. It correlated with stemness TFs, sphere growth, and a unique proteomic pattern dominated by induction of p21, LEF1 and SOX9. Further characterization of the two distinct responder phenotypes is ongoing. Conclusions. Our systematic approach exposed the structure of drug sensitivity in glioblastoma and indicates a distinct subclass vulnerable to proteasome inhibition. Next generation proteasome inhibitors with reduced CNS toxicity may have therapeutic relevance in this set of glioblastoma.

SIMULTANEOUS ACTIVATION OF SHH- AND WNT-SIGNALING IN NEURAL PROGENITORS DRIVES FORMATION OF EMBRYONAL TUMORS WITH MULTILAYERED ROSETTES (ETMR) AND INDICATES POTENTIAL THERAPEUTIC AVENUES

Julia E. Neumann, Annika K. Wefers, Sander Lambo, Edoardo Bianchi, Marie Bockstaller, Mario M. Dorostkar, Valerie Meister, Pia Schindler, Andrey Korshunov, Katja von Hoff , Johannes Nowak, Monika Warmuth-Metz, Marlon R. Schneider, Ingrid Müller-Renner, Daniel J. Merk , Mehdi Shakarami, Rainer Glass, Jennifer A. Chan, M. Mark Taketo, Philipp Neumann, Marcel Kool and Ulrich Schüller
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Embryonal Tumors with Multilayered Rosettes (ETMRs) have recently been described as a new entity of rare pediatric brain tumors with fatal outcome. We show here that the overexpression of Lin28A, which is a hallmark of human ETMRs, augments Sonic Hedgehog (Shh)- and Wnt-signaling through the regulation of let7-miRNAs. The simultaneous activation of Shh- and Wnt-signaling in turn is sufficient to induce ETMRs from neural precursors of the murine cortical subventricular zone (SVZ). These tumors are well responsive to the Shh-inhibitor Arsenic trioxide (ATO), and the treatment of immunocompromised mice with orthotopically injected human ETMR-cells finally demonstrates that inhibition of Shh-signaling may serve as a therapeutic option for patients with ETMRs.

MODELLING MOLECULAR SUBTYPES OF MEDULLOBLASTOMA IN XENOPUS TROPICALIS USING CRISPR/CAS9

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Unit of Developmental Biology, Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium

Medulloblastoma is the most common malignant brain tumor in children. It affects the posterior cranial fossa, which consists of the cerebellum and brainstem. Current therapy is mainly limited to surgical resection, craniospinal radiation and chemotherapy. Even though long term survival rates can be high, patients suffer from significant morbidity, including neurocognitive impairment and endocrine dysfunction, as a result of the aggressiveness of the treatment options. Recently, genomic approaches revealed four distinct medulloblastoma subtypes based on molecular markers underlying tumorigenesis: Wnt, Shh, group3 and group 4. This has led to adjusted risk stratification with Wnt and group 3 subtype tumors showing best and worst prognosis respectively. Moreover it has provided a platform for research into molecular targeted therapies, which could greatly diminish disease sequelae. We employed genome editing via CRISPR/Cas9 in Xenopus tropicalis to model Wnt-type and Shh-type medulloblastoma through apc and patched1 inactivation respectively. We believe these models are ideally suited to elucidate the role of candidate tumor modulators through gRNA multiplexing. Furthermore we want to use CRISPR/Cas9 mediated genome editing to develop representative group 3 and 4 medulloblastoma models.

IL-6: KEY MODULATOR IN GLIOMA IMMUNE MICROENVIRONMENT

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The microenvironment of glioblastoma (GBM) is a main cause for the ineffectiveness of current multimodal treatments. This microenvironment is populated by infiltrating immune cells such as glioma-associated microglia/macrophages (GAMs). The secretome of this immune microenvironment (IM) regulates cell-cell response as does the secretome of tumor cells. GBM-derived signals contribute to IM modulation by reprogramming GAMs to acquire a functional phenotype supporting tumor growth and invasion. For instance, microglia (MG) can be polarized from the pro-inflammatory (M1-like) to a cytoprotective (M2-like) phenotype by tumor environmental cues. The identification of key molecules that regulate this polarization is thus an interesting approach to understand GBM-immune cell complementary symbiosis. We aimed to identify key regulators by analyzing the IM secretome when exposing GBM cells to an immunotherapeutic strategy based on M1-like modulation of microglia. We used an in-vitro three-dimensional model consisting of spheroids embedded in a collagen matrix. Immune modulation by GBM cells and their capacity to invade in absence or presence of M1-like or untreated MG present in the matrix was studied using spheroids of murine SMA-560 astrocytoma cells mixed with murine primary untreated MG. Collagen-implanted M1-like MG appeared to inhibit growth of spheroids at early time points but were later supporting this invasion, suggesting M2-like polarization by tumor cells. Secretome analysis indicated interleukin-6 (IL-6) as a key candidate of this polarization. Indeed IL-6 neutralization successfully restored an M1-like functional profile. We are currently evaluating our working hypothesis on the IL-6/STAT3 signaling pathway as an important inducer of tumor cell invasion via enzymatic activity of MMPs. Identification of the extracellular tumor substrate molecules may facilitate the development of more efficient glioma immune-therapy.

NEW PRECLINICAL MODELS FOR NEURO-ONCOLOGY: INTEGRATING DATA FROM PD3D® MODELS AND ORTHOTOPIC XENOGRAFTS.

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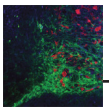
Glioblastoma multiforme (GBM) is the most common, aggressive malignant brain tumor in adults and with a mean survival time between 8 and 18 months, current standard care is of limited benefit.



To support basic and translational research, 3D cell cultures (PD3D) and orthotopic (i.cer.) patient-derived xenografts (PDX) models are of increasing importance and represent an essential tool in GBM research. These models help identifying novel targets and biomarkers and eventually guide personalized therapies. We have recently established in parallel 4 PD3D, 23 s.c. and 9 i.cer. PDX models from GBM tissue. Primary GBM tissue was processed for PD3D and PDX establishment. In brief, for PD3D cells were grown as organoids in a scaffold. For s.c. models GBM tissue was directly transplanted to immunodeficient mice. To mimic tissue-specific interactions of human GBMs with the host microenvironment we performed i.cer. transplantation. PD3D and PDX tumor tissues were characterized by IHC and PanelSeq. PD3D cytotoxicity was determined by CTG. IC50 values ranged in the order of clinical plasma concentrations. Both (s.c. and i.cer.) PDX models were screened for sensitivity towards drugs known to pass the blood-brain-barrier including temozolomide (TMZ) in mono- and combination-therapy. In retrospective, PD3Ds have proven useful for guiding in vivo screens. Response in PDX glioma models is highly heterogeneous. Most models show a strong initial sensitivity to TMZ (18/23). In s.c. PDX models, the strongest response was induced by bevacizumab (7/21), irinotecan (15/21), and TMZ (16/21), whereas the other drugs were significantly less effective. In combination therapies treatments with TMZ plus another drug eventually lead to beneficial synergistic effects. In contrast, first data indicates reduced sensitivity to treatments comparing i.cer. to s.c. transplantation in the same PDX model with bevacizumab, irinotecan and everolimus less active in the i.cer. model.

CONDITIONALLY PROLIFERATING PRIMARY MURINE ASTROCYTES RECAPITULATE CHARACTERISTICS OF HUMAN GLIOBLASTOMA

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Despite considerable advances of research on gliomas these tumors still bear a grim prognosis. Animal models that closely reproduce the biological characteristics of gliomas might lead to a better understanding of glioma pathogenesis and could assist in the development of novel therapeutic modalities. Most available animal models of gliomas reflect the human disease only partially. In particular the hallmark of human astrocytomas, the infiltration of the surrounding brain tissue, is rarely seen in animal models. The aim of the present study was to generate conditionally proliferating murine astrocytic tumor cells in order to establish an animal model that reproduces the relatively slow proliferation rate of human glioma cells and to investigate whether slow proliferation correlates with infiltrative behaviour. Primary murine p53-/- astrocytes were transduced with two oncogenes 1. constitutively active myr-Akt and 2. c-myc under control of a Tet-Off-System.The characteristics of the resulting cells were investigated in vitro and in vivo in the brain of C57BL/6 mice. Transduced astrocytes developed a tumor cell phenotype as reported previously and in addition revealed conditional proliferation depending on c-myc expression for prolonged periods of time. Moreover, proliferation could be repeatedly switched ON and OFF in vitro. Transduced astrocytes were stereotactically implanted into the brain of congenic mice and the size of the resulting tumors could be regulated by feeding the mice tetracycline. The macroscopic and histological appearance of the tumors depended both on the number of passages in vitro and the duration of the growth in vivo. Early passage cells with slower proliferation in vitro induced only microscopically visible tumors in the brain, while cells from later passages lead to macroscopically visible tumors in the same period. Histologically, the murine tumors from early passages in vitro revealed highly polymorphic cells similar to human glioblastoma. Occasionally, infiltration of the surrounding brain was observed. Together, we present a novel mouse model of astrocytic tumorigenesis with controllable proliferation that might be useful in the further study of gliomagenesis and glioma therapy.

INSIDIA: IMAGEJ MACRO FOR HIGH-THROUGHPUT AND HIGH-CONTENT SPHEROID INVASION ANALYSIS

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Time-series image capture of in-vitro 3D spheroidal cancer models embedded within an extracellular matrix affords examination of spheroid growth and cancer cell invasion. However, a customisable, comprehensive and open source solution for the quantitative analysis of such spheroid images is lacking. Here, we describe INSIDIA (INvasion SpheroID ImageJ Analysis), an open-source macro implemented as a customisable software algorithm running on the ImageJ platform, that enables high-throughput high-content quantitative analysis of spheroid images (both bright-field grey and fluorescent images) with the output of a range of parameters defining the spheroid 'tumour' core and its invasive characteristics.

SPROUTY2 ENHANCES TUMOR-PROPAGATING POTENTIAL OF GLIOMA CELLS

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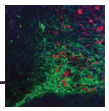
Background. In numerous malignancies including glioblastoma multiforme (GBM), a subpopulation of stem-like cells greatly influences tumor biology by controlling proliferation dynamics as well as the response to chemotherapy. Sprouty2 (SPRY2), a feedback regulator of receptor tyrosine kinase (RTK) signaling, has been shown to be associated with drug resistance in GBM, thus raising the possibility that SPRY2 may have a critical function in stem-like GBM cells. Methods. SPRY2 expression in GBM with low-grade gliomas or non-tumor tissues was compared using microarray analysis. Effects of gain- and loss-of-function of SPRY2 to regulate the tumorigenic capacity of established GBM cell lines were analyzed using sphere formation assays and human xenograft models. Results. SPRY2 was up-regulated in malignant gliomas and correlated with reduced survival in the glioma patients. SPRY2 knockdown significantly impaired neurosphere formation of glioma cells. Consistent with these findings, SPRY2 was required for tumor propagation in vivo. In addition, we demonstrate that SPRY2 induced VEGF expression in glioma cells, which was accompanied by increased blood vessel formation in GBM xenografts. Conclusions. The present study highlights a tumorigenic potential of SPRY2 that is based on its capacity to regulate the stemness phenotype and neovascularization, suggesting that SPRY2 regulated signaling pathways may be a promising pharmacological target for GBM.

KCA1.1 CHANNEL AUXILIARY BETA SUBUNIT COMPOSITION IN GLIOBLASTOMA MULTIFORME

Zoltán Pethő1, Álmos Klekner3, Lászlo Bognár3, Zoltán Varga1,2, György Panyi1

Department of Biophysics and Cell Biology, University of Debrecen, Hungary, 2) MTA-DE-NAP Ion Channel Structure-Function



Research Group, RCMM, Debrecen, Hungary 3) Department of Neurosurgery, University of Debrecen

Glioblastoma Multiforme (GBM) is the most aggressive glial cancer as well as the most common primary malignant brain tumor. Even in the 21st century, regardless of aggressive treatment, the median survival remains less than two years following diagnosis. GBM tumor cells and model systems express KCa1.1 (BK, Slo1) as their major K+ channel. Moreover, it is known that auxiliary β and γ subunits modulate the biophysical properties of the KCa1.1 channel. In this study, our aim is to characterize the KCa1.1 β subunit composition in both tumor model systems and in freshly isolated tumor cells. We used the cultured tumor cell line U87-MG and freshly isolated tumor samples obtained from the Neurosurgery Department of the University of Debrecen. Following isolation, grade IV GBM samples were examined by rt-PCR, confocal microscopy for glial markers and whole-cell currents using patch-clamp technique. In our patch-clamp experiments of GBM cells we measured the activation and inactivation kinetics of the KCa1.1 channel as well as the pharmacological responses to various KCa1.1 modulators (e.g. arachidonic acid, paxilline). In accordance with current literature, we observed KCa1.1 current on GBM cells showing inhibition by paxilline. Moreover, we could deduce the presence of functional β subunits based on rt-PCR, current kinetics and pharmacological response. In conclusion, our findings also support that KCa1.1 has an important role in GBM pathogenesis and the inhibition of this channel specifically targeted via auxiliary subunits may have a potential therapeutic consequence in the future.

Funding sources: KTIA_NAP_13-2-2015-0009; NFKI-6_119417

CHARACTERIZATION OF MICROGLIA/MACROPHAGE PHENOTYPES IN GLIOBLASTOMA PATIENT-DERIVED XENOGRAFT MOUSE MODELS

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A major contributing factor to glioblastoma (GBM) development and progression is its ability to evade the immune system. Malignant gliomas release factors that recruit resident microglia, macrophages and other peripheral immune cells to the tumor site and transform them into tumor-supportive cells. Here, we investigate the contribution of tumor-associated microglia/macrophages (TAMs) in intrinsic tumor escape mechanisms by analysing their phenotypic and functional adaptation in the tumorigenic process. Specifically, we aim to elucidate the variation in molecular profiles of TAMs during tumor progression and with respect to different histopathological GBM features, such as invasion and angiogenesis. For this, we combine immunohistochemistry with multicolor flow cytometry analysis to characterize TAM signatures in three different orthotopic patient-derived xenograft (PDX) mouse models which, in our hands, represent different stages along tumor progression as well as recapitulate strong inter- and intra-tumor heterogeneity present in patients. 'Invasive' xenograft GBM tumors were characterized by a highly invasive and infiltrative tumor growth pattern with an apparent normal brain vasculature, while 'angiogenic' phenotypes displayed a clear demarcation with necrotic areas and microvascular proliferation. 'Intermediate' tumors shared features of both invasion and vessel abnormalities. Iba1-positive cells showed inter- and intra-tumor regional heterogeneity, displaying different morphologies in the tumor core, the infliltration zone and the distal area. Differing composition of CD11b-positive myeloid cell constituents and their immune phenotypes reflected histological tumor hallmarks. The analysis of our PDX models shed light on spatial and temporal he terogeneity of TAM polarization in GBMs. These analyses pave the way to novel targeted immune-therapeutic approaches to be tuned according to tumor stage and histopathology.

IMIDAZOLE CONTAINING COMPOUNDS ARE POTENTIAL DRUGS FOR THE TREATMENT OF GLIOBLASTOMA

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Carnosine (beta-alanyl-L-histidine), a naturally occurring dipeptide, inhibits the growth of glioblastoma cells. As L-histidine mimics the anti-neoplastic effect, we investigated whether the release of L-histidine from carnosine is required for that. Glioblastoma cell lines and primary cultures were exposed to carnosine or L-histidine in the presence of the carnosinase inhibitor bestatin. Cell viability was analyzed by cell based assays and carnosinase expression was determined by immunoblotting and qRT-PCR. Intracellular amounts of carnosine and L-histidine were determined by Liquid Chromatography coupled to Mass Spectrometry. 48 hour exposure to carnosine (50 mM) significantly reduced viability in all tumor cells to an average of 73.6±20.5%, whereas L-histidine revealed a more pronounced effect (49.8±18.6%). We observed a significantly enhanced ($p<0.05$) abundance of L-histidine in 9 of 10 cell lines and in 4 of 5 primary cell cultures exposed to carnosine. However, no correlation between the release of L-histidine or the expression of carnosinases and the anti-neoplastic effect was observed. Furthermore, the aminopeptidase inhibitor bestatin did neither attenuate nor enhance the effect of carnosine. These observations indicate that the release of L-histidine from carnosine is not required for its anti-neoplastic effect although L-histidine revealed a more pronounced effect on viability than carnosine. As L-histidine and most likely its imidazole ring appear to be responsible for growth-inhibition, this observation can be considered for the design of potential drugs that are able to deliver therapeutic amounts of imidazole groups to tumors.

MORPHOLOGICAL AND ADHESION DIVERSITY OF PRIMARY GBM

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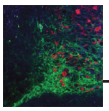
Patients' responses to the therapeutic inhibition or activation of integrin aimed at reducing or enhancing adhesion respectively may depend on the mechanical properties of the tumour cell population and its adhesion to the surrounding extracellular matrix. Given the phenotypic diversity and the infiltrative-dispersive nature of GBM, there is likely to be a variation in adhesion strength across individual tumours (different patients) and between cell lines within the same tumour. We measure cell-matrix adhesion in cell populations, accounting for GBM heterogeneity by simultaneously treating a large number of cells. Patient-derived cell lines are characterised with respect to their location within the tumour and their invasive/infiltrative capacity. We employ dual-mode reflectance and fluorescence confocal laser scanning microscopy to measure cell volume, area and cell-matrix contact area in live cells. GBM cell migration is measured by tracking cells for 48 hours using time-lapse microscopy. Initial findings from our pilot study suggest a diverse morphology (cell area and contact/adhesion area) between tumours. Adhesion area was inversely correlated with GBM migration. The relationships between cell-matrix adhesion strength, morphology and migration



will be investigated to show whether adhesion strength can be used as predictor of GBM invasiveness and infiltrative capacity. We will also build on this model to assess the impact of the morphological and adhesion diversity of primary GBM on the cellular response to chemical and immunotreatments.

GENETIC ANALYSES ON GLIOBLASTOMA STEM LIKE CELLS AND GLIOBLASTOMA TISSUE USING SNP ARRAY AND GENE EXPRESSION

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Only few information exist about comparative genetic data of glioblastoma stem like cells (GSCs). Genetic analyses on GSCs and glioblastoma tissue using SNP array and gene expression were performed to get more information about glioblastoma. Serum-free cell subpopulation was isolated by the markers CD133 and CD15 through multi-parameter magnetic-activated cell sorting technique. Peripheral blood, tumor tissue, serum-free culture, and the isolated cell subpopulations were analyzed by SNP array and gene expression. For preliminary characterization of GSCs, we confirmed the stem cell features of GSCs in the serum-free culture by the expression of Nestin, SOX2, and CD133 applying immunofluorescence staining. SNP array analyses showed unique genetic profile between tumor tissue and cell subpopulations, e.g. gain of chromosome 7, partial and/or complete loss of chromosome 10. Furthermore, we detected distinct differences in the genetic profile between tumor tissue and cell subpopulations, e.g. loss of chromosome 4, and segmental uniparental disomy of 9p24.3->p21.3, only in glioblastoma stem like cell subpopulations. Gene expression analyses showed considerable differences between tumor tissue and serum-free cultures. In comparison between tumor tissue vs CD133+/CD15+ cells, we detected strong up- and downregulated genes in tumor tissue compared to CD133+/CD15+ cells. Whereas 418 genes were upregulated in tumor tissue, 44 genes were downregulated in tumor tissue comparing to CD133+/CD15+ cells. Pathway analyses showed that upregulated genes in CD133+/CD15+ cells in comparison to tumor tissue have mostly influence on regulation of cell cycle processes and cancerogenesis. In contrast, upregulated genes in tumor tissue compared to CD133+/CD15+ cells may influence pathways of immunity and diseases due to immunodeficiency. In summary, we detected some minor differences when comparing GSCs and tumor tissue using SNP array and gene expression.

CHARACTERIZATION OF GLIOBLASTOMA CELL INVASION: TOWARDS NOVEL THERAPEUTIC TARGETS

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A major hallmark contributing to the aggressive potential of glioblastoma (GBM) is its highly invasive behaviour. Since invasive cells cannot be easily removed by surgery or irradiation, GBM always recurs and is eventually lethal. Therefore it is crucial to fully understand the invasion process of GBM and identify key molecules driving invasive properties that may represent new therapeutic targets to treat GBM patients. In this project, the invasive behaviour of patient-derived GBM cell lines grown as neurospheres was characterized using in vitro invasion assays, ex vivo brain slice cultures and in vivo orthotopic xenografts in mice. GBM cell lines grown as 3D neurospheres displayed variable but reproducible degrees of invasion when compared to each other. The in vitro and ex vivo invasive potential correlated well with their behaviour in vivo. E.g. the cell line, which developed a confined, poorly invasive tumor in vivo showed the least invasion in culture, while the cell lines infiltrating the whole brain parenchyma in vivo also demonstrated highest invasion scores in vitro and ex vivo. Thus, the in vitro and ex vivo invasive behaviour of patient-derived GBM cell lines is mirrored by the in vivo invasive characteristics. To identify candidate genes responsible for invasion in GBM, a whole genome library shRNA interference screen was performed and the most promising candidate was validated in invasion assays, confirming its role in invasion in GBM cell lines. The candidate gene identified by the shRNA interference screen has demonstrated invasive capacities in vivo reflecting a major GBM hallmark and therefore represents a novel and very promising target to overcome obstacles in GBM treatment.

SPONTANEOUS CA2+ TRANSIENTS IN MOUSE MICROGLIA.

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Microglia are the resident immune cells in the central nervous system and many of their physiological functions are known to be linked to intracellular calcium (Ca2+) signaling. We demonstrate that isolated and purified mouse microglia -either freshly isolated or cultured - display spontaneous and transient cytosolic Ca2+ elevations lasting for around ten to twenty seconds and occurring at frequencies of around five to ten events per hour and cell. The events were absent after depletion of internal Ca2+ stores, by phospholipase C (PLC) inhibition or blockade of inositol-1,4,5-trisphosphate receptors (IP3Rs), but not by removal of extracellular Ca2+, indicating that Ca2+ is released from endoplasmic reticulum intracellular stores. We furthermore provide evidence that autocrine ATP release and subsequent activation of purinergic P2Y receptors is not the trigger for these events. Spontaneous Ca2+ transients did also occur after stimulation with Lipopolysaccharide (LPS) and in glioma-associated microglia, but their kinetics differed from control conditions. We hypothesize that spontaneous Ca2+ transients reflect aspects of cellular homeostasis that are linked to regular and patho-physiological functions of microglia.

ROLE OF CXCL8-CXCR1/2 AXIS IN GLIOBLASTOMA CELL PROLIFERATION, INVASION AND VASCULAR MIMICRY.

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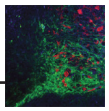
Despite intense molecular characterization and research in finding novel therapeutic targets, glioblastoma remains most lethal tumor of central nervous system. Among many contributors in gliomagenesis, chemokines have drawn special attention due to their involvement in plethora of biological processes. In present work, we examined the differential gene expression of all chemokines and their receptors among low grade (DA) and high grade astrocytoma (GBM) to identify pro- glioblastomagenic chemokine axis. In vitro targeting of the key axis identified revealed important discernments. Gene expression was studied using RT2 ProfilerTM PCR array in Diffuse Astrocytoma and GBM tissue samples. Gene ontology analysis was performed to funnel down important axis in GBM followed by validation at protein



level using tissue microarray. Chemokine axis identified was targeted in vitro by neutralizing antibodies and chemical antagonist to see the impact on cell proliferation, clonogenic survival, multicellular tumor sphere formation and spheroid invasion. The differential gene expression and gene ontology analysis revealed CXCL8 as an important chemokine associated with GBM progression. Further, localization of CXCL8 and its receptors suggested possible autocrine and paracrine signalling promoting tumor cell proliferation, neovascularisation and possibly vascular mimicry in GBM. Effective inhibition of CXCL8- CXCR1/2 axis led to significant reduction in cell proliferation, clonogenic survival and invasion in U-87MG and LN-18 cell lines. These results suggest targeting CXCL8-CXCR1/2 axis impacts GBM cell proliferation and invasion which requires further in vivo investigation to confirm its therapeutic potential in GBM.

SLOW DIVIDING GLIOBLASTOMA STEM CELLS DEPEND ON LIPID METABOLISM AND MITOCHONDRIAL FUNCTION

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1Cardiff University School of Biosciences, European Cancer Stem Cell Research Institute; 2Dept Neuroscience, University of Florida; 3Dept Neurosurgery, University of Florida; 4Dept Pathology, Immunology and Laboratory Medicine, University of Florida

Current cancer therapeutics effectively eliminate rapidly proliferating cells, but spare slowly dividing populations. Expansion of these therapy-selected slow-cycling cells (SCCs) might therefore contribute to tumor relapse. We previously found that SCCs exhibit cancer stem cell characteristics and therapy resistance in glioblastoma (GBM). Here, we show that proliferative GBM cells utilize aerobic glycolysis, while SCCs rely on oxidative phosphorylation (OxPhos) to meet energy demands. Metabolomics studies confirmed heterogeneity in GBM with differential metabolic signatures between fast and slow-dividing cells. TCGA analysis of primary and recurrent GBM revealed an overlap between signature metabolic pathways in SCCs and recurrent GBM. The reliance of SCCs on OxPhos reveals a targetable vulnerability, which may be exploited to eradicate this treatment-resistant population. Therefore, we tested the efficacy of metabolic inhibition in GBM. Although SCCs were less sensitive to glycolysis inhibition, they showed sensitivity to OxPhos blockade. Importantly, dual inhibition of glycolysis and mitochondrial respiration was effective at targeting both metabolically distinct populations and preventing metabolic adaptation. These findings indicate the existence of metabolic heterogeneity in GBM, and suggest that subpopulations of treatment-resistant cells, which contribute to relapse, may rely on OxPhos rather than aerobic glycolysis, revealing susceptibilities that could be exploited therapeutically.

THE MIR-143/145 CLUSTER AS CRITICAL REGULATOR IN GLIOMAGENESIS?

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Objective: MicroRNAs as crucial posttranscriptional modulators of carcinogenesis have been studied extensively recently. Their integratory function in numerous pathophysiological intracellular processes has made them a most promising target for modern anticancer therapy. Being aware of that, the aim of our analysis was to investigate the expression patterns of mTOR- and associated microRNAs in gliomas. Methods: The expression of mTORC1 associated RAPTOR, mTORC1 effectors S6K and 4E-BP1 as well as mTORC2 associated RICTOR and FoxO 1 were quantitatively analyzed in 50 glioma specimens (WHO II°-IV°). Additionally, the expression of microRNAs (miR-99a, miR-99b, miR-100 and miR-101, miR-143 and miR-145) associated with different components of the mTOR-network were quantitatively analyzed in 18 glioma specimens (WHO II°-IV°) using realtime polymerase chain reaction (qPCR); these were subsequently evaluated applying the comparative ΔΔCt method against healthy brain tissue. Results: In all glial tumor entities components associated with the mTORC2-AKT-FoxO-axis were expressed preferentially. Furthermore, the expression of miR-143 and miR-145 was significantly decreased. Conclusions: Analyzing mRNA and microRNA expression profiles of gliomas (WHO II°-IV°) correlated to the mTOR-network, we detected a statistically significant expression of mTORC2-associated components. Moreover, the significant minor expression of microRNA 143 and 145 suggests that this miR-cluster plays a relevant role in the mTORC2-AKT-FoxO-axis. Therefore functional analyses to elucidate this regulatory mechanism should be pursued in further investigations.

DIFFERENTIAL EFFECTS OF SMAC MIMETIC GDC-0152 IN GLIOBLASTOMAS DEPENDING ON TUMOR MICROENVIRONMENT

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Glioblastomas are highly aggressive tumors. Relapses and tumor regrowth after treatment are driven by cancer stem cells (CSC). Therefore targeting CSC represents a major challenge to improve GBM bearing patient's survival. One strategy to eliminate CSC is to trigger their differentiation. To this purpose we used small molecules Smac mimetics which antagonize inhibitor of apoptosis proteins. Smac mimetics in non-apoptotic conditions were able to differentiate CSC in less tumorigenic astrocytic cells in a NF-kB dependent manner1. As GBM are highly hypoxic tumors and hypoxia can induce treatment resistance, we asked whether Smac mimetic GDC-0152 have the same action on CSC in their microenvironment. To this end we placed CSC in hypoxia (2% O2) for 8 days to analyze GDC-0152 impact on inhibitor of apoptosis proteins expression, differentiation, cell viability, apoptosis and proliferation. Surprisingly, in hypoxia GDC-0152 was more effective to inhibit inhibitor of apoptosis proteins expression than in normoxic conditions. Although GDC-0152 induced stem cell differentiation in normoxia, this was not recorded in hypoxic conditions. In contrast, drastic increase of apoptosis and decrease of cell proliferation were reported. Indeed, GDC-0152 significantly decreased cell viability at nanomolar doses only in hypoxia. The signaling pathways involved in hypoxia upon GDC-0152 treatment are under investigations. This work shows that GDC-0152 triggers anti-tumoral effects depending on tumoral microenvironment: in normoxia it induces GBM stem cell differentiation and in hypoxia it is a more effective cytotoxic drug. Therefore, smac mimetic GDC-0152 represents a promising treatment for patients bearing GBMs.

1 Tchoghandjian et al., CDD, 2014

TARGETING HEPARAN SULFATE IN THE BRAIN TUMOR MICROENVIRONMENT

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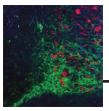
Brain Tumours are invasive and they notably infiltrate the brain parenchyma. This is the main reason they remain fatal despite resection as cells have already migrated away, leading thus to



rapid regrowth of the tumour. Hence, the brain tumour microenvironment plays a crucial role in the regulation of this process. In this study, we investigate the role of heparanase (HPSE) enzyme in pediatric brain tumours which is an endo-β-D-glucuronidase has a crucial mission in pediatric brain tumour aggressiveness. Heparan sulfate proteoglycans (HSPGs), omnipresent components of the extracellular matrix of the brain, modulate the activities of other factors such as cytokines, growth factors etc. The major enzyme that cleaves HS, heparanase (HPSE), is an important regulator of ECM remodelling and has recently been shown by our lab to promote the growth of glioma, and correlate to patient survival in GBM. We now report that expression of HPSE is higher in pediatric tumours than in healthy brain tissue. We found that active or/and latent form of HPSE stimulate proliferation of MB cells and PNET (cell line PFSK, previously classified as PNET). Pediatric brain tumour cells when treated with recombinant HPSE (rHPSE) in the form of latent 65kDa, which requires intracellular activation, increased the number of viable cells, and interestingly, rapidly activates ERK and AKT pathways, even before enzymatically active HPSE was detected. Thus, rHPSE has direct effects on MB and PFSK cells. Furthermore, we used a HPSE inhibitor (PG545) that efficiently killed pediatric brain tumour cells but not normal human astrocytes, and was found to potently reduce the size of flank tumours derived from these tumour cells. Heparanase has been associated to increased cancer metastasis, angiogenesis and significantly reduced post-operation survival of patients. Our data suggest that HPSE is actively involved in pediatric tumor progression and constitutes a promising target for drug development.

COMPARING EXPRESSION ANALYSIS OF THE KEY PROTEINS OF THE PI3K/MTOR-SIGNALING PATHWAY IN HUMAN MENINGIOMA AND GLIOMA

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Aims: The PI3K/mTOR pathway is a critical signal transduction system linking oncogenes and multiple receptors to cellular functions like proliferation, quiescence and longevity. We analyzed the PI3K/mTOR pathway in human high-grade gliomas (WHO°III-IV) and meningiomas (WHO°I and II-III) to analyze if the pathway is switched on or off by comparing gene and protein expression between the WHO grades within one tumor entity as well as between the two tumor entities. Methods: For quantifying the expression of specific key genes of the pathway (IGF1-R, IRS1, PI3K, PTEN, RHEB, RPTOR, RICTOR, FKBP12 and TBC1D7) we isolated mRNA from 56 gliomas and 54 meningiomas, analyzed it with RT-qPCR and quantified with efficiency based- and ΔΔCt-method. Furthermore we obtained the protein from the samples and performed western blotting analysis to get the protein expression. Results: The expression analysis showed differences in single genes (RHEB, PI3K) in gliomas compared to meningiomas. In gliomas itself we detected significant differences in IGF1-R and RHEB, in comparison to normal tissue. In meningioma the gene expression compared to normal tissues was significant in both grade groups in IGF1-R and PTEN, in FKBP12 in group °II-III and in RICTOR in °I. The protein expression in gliomas was like we expected from the literature. In meningioma we established a signal but no significant differences between the grades in the proteins p-IGF1-R, p-IRS1, PI3K, PTEN, RHEB, RPTOR and RICTOR. Conclusions: The PI3K/mTOR signaling pathway in ectodermal gliomas is genetically up regulated, but protein-wise deactivated. In mesodermal meningiomas we could only detect genetic expression changes compared to non-pathological control tissue.

ROLE OF GDF-15 IN HIGH MALIGNANT GLIOMA

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High grade glioma constitutes the prevalent and most malignant primary brain cancer in humans with a very poor prognosis. The tumors are characterized by a high proliferative rate, aggressive invasiveness and marked therapy resistance enabling tumors to escape complete surgical resection, chemo- and radiotherapy. Growth differentiation factor-15 (GDF-15) is a distant member of the TGF-beta superfamily which is implicated in diverse pathological processes including atherosclerosis, heart failure and cancer. In high grade glioma patients, high expression of GDF15 correlates with shorter overall survival. So far most studies addressed the role of GDF15 in tumor cells, although high expression of the protein is also observed in the tumor microenvironment (TME) i.e. in tumor-associated reactive astrocytes and in infiltrating immune cells. To decipher the in vivo role of TME-derived GDF15 in high-grade glioma, we stereotactically implant GDF15-positive and negative syngeneic glioma cells with different invasive capacities into wt and GDF15-deficent C57BL/6 mice. The resulting tumors are analyzed at defined time points for histopathology, tumor size, invasion, and proliferation as well as the extent of reactive gliosis and the recruitment of immune cells into the tumor tissue. Taken together these analyses will help to define whether therapeutic approaches based on functional ablation of GDF15 may constitute a promising strategy for the treatment of high grade glioma.

OPEN CHROMATIN AND EPIGENETIC LANDSCAPE OF HUMAN BENIGN AND MALIGNANT GLIOMAS

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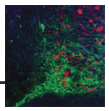
Gliomas, the most common primary brain tumors, are clinically divided into 4 grades according to their aggressiveness, with benign pilocytic astrocytomes being grade I and glioblastoma (GBM) grade IV being the most aggressive malignancy. Despites aggressive conventional treatments GBM is incurable and a mean survival is 14 months. Recently, a growing number of evidence indicates dysregulation of epigenetics in gliomas as a driving force of transcriptional changes and a pathogenic mechanism. With epigenetic profiling in cancer now available, we sought to map patterns of open chromatin and active transcription contributing to gliomagenesis. In the present study we report the first results of large-scale epigenomic profiling of low and high grade gliomas. In order to generate an advanced map of regulatory elements in gliomas, we collected fresh glioma samples obtained as surgical resections and performed multilayer genome-wide analyses on the same sample to map: 1) chromatin accessibility, 2) open chromatin regions, 3) histone modifications, 4) transcribed regions. To achieve these goals, we have optimized protocols of tumor tissue processing for ChIP-seq (mapping of histone marks), high resolution DNase I seq and ATAC-seq (mapping of DNase I hypersensitive sites and accessible chromatin). Obtained data are intersected with a global transcriptome analysis (RNA- seq). Generated high-quality data are comparable to publicly available data from Roadmap Epigenomics database and show that most of the regulatory regions are highly conserved. We present results of bioinformatic analysis of intersection of H3K4me3 ChIP-seq and DNase I seq data that identify active promoters. Moreover, we identify regulatory regions altered in glioblastoma. This is the first identification of open chromatin areas and gene regulatory



networks in low and high grade gliomas which may provide new insights into mechanisms of glioma development and epigenetic disturbances in gliomas.

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ANALYSIS OF IDH1 AND L-PLASTIN IN HUMAN MICROGLIA/MACROPHAGES EXPOSED TO GLIOBLASTOMA CELLS

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Brain resident microglia and infiltrating blood-borne macrophages contribute significantly to the heterogeneity of the glioblastoma (GBM) mass and support tumor growth and invasion. Manipulation of these two populations of immune cells, collectively known as tumor-associated microglia/macrophages (TAMs), represents a promising therapeutic strategy that might improve the current treatment options, which mainly target tumor cells. The design of such alternative strategies requires a better understanding of the interactions between tumor cells and TAMs. A quantitative proteomics study using the stable isotope labeling with amino acids in cell culture (SILAC) approach combined with LC-MS/MS was performed with TAMs exposed to GBM cells (set 1) or to control human astrocytes (set 2) in the indirect co-culture Transwell system. We used a human induced pluripotent stem cell-derived microglia (iPSdM) cell line as a substitute for human TAMs. Outcomes from the cytosolic fraction analysis were compared between the two sets. Differential protein expressions relevant for energy production and cytoskeletal activity were observed. Validation was undertaken for L-Plastin (LCP1) and isocitrate dehydrogenase 1 (IDH1), which showed a decreased expression in iPSdM exposed to GBM cell lines. LCP1 is an actin-bundling protein and an abundant component of podosomes structures, which are essential for cell motility and invasion. IDH1 is a significant provider of α-ketoglutarate and NADPH, thus contributing to both the citric acid cycle and mitigation of oxidative damage. Data on gene and protein expression in cells as well as in GBM tissues will be presented and discussed in regards to TAMs functions in GBM biology.

BIDIRECTIONAL NEURON-GLIOMA INTERACTIONS: EFFECTS OF GLIOMA CELLS ON SYNAPTIC ACTIVITY AND ITS IMPACT ON TUMOR GROWTH

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Gliomas are the most common primary brain tumors of the CNS. The development of novel therapies requires a better understanding of the biology of glioma cells and their interactions with resident cells. Evidences of highly disabling peritumoral dysfunctions in patients with glioma and data showing that levels of activity in peritumoral areas can control glioma cell proliferation, suggest a bidirectional interplay between the tumor and surrounding brain tissue. Little is known about functional changes during glioma growth and investigations on the role of neural activity in glioma have yielded contradictory results. Here we provide a detailed understanding of how tumor growth reverberates on the function of neuronal networks and how it is influenced by neural activity. We injected GL261 cells into the primary visual cortex of syngeneic C57JBl6 mice and we monitored neural response using chronic recordings of visual evoked potentials (VEP) during glioma growth. We observed an initial progressive increase in VEP amplitude, indicative of stimulus-dependent response potentiation (SRP). This phase was followed by a rapid decay and deterioration of visual responses, that completely disappeared by day 25 after cell transplant. To investigate the role of neural activity in controlling glioma progression we manipulated neural activity and we analyzed glioma cell proliferation. In particular, we used botulinum neurotoxin A (BoNT/A) to block synaptic activity and visual deprivation (dark rearing, DR) to reduce physiological activity. We found that both BoNT/A and DR lead to a higher density of proliferating cells indicating that neural activity restrains glioma proliferation. All together, these findings demonstrate that glioma impacts on the surrounding neurons reducing visually-evoked activity. This reduction, in turn, promotes glioma proliferation and might trigger a feedback loop that exacerbate glioma progression.

SPROUTY2: AN IMPORTANT MODULATOR OF SIGNALING AND FGF RECEPTOR TRAFFICKING IN GLIOBLASTOMA MULTIFORME

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Introduction: Sprouty2 (SPRY2), the strongest negative regulator of the RAS/ERK pathway, acts as tumor suppressor in many types of cancer. Recent evidence indicated that SPRY2 is also involved in receptor tyrosine kinase transport. Here, we investigated the effect of SPRY2 on activation of two major singaling pathways (ERK, AKT) and intracellular trafficking of fibroblast growth factor receptor type 1 (FGFR1) in the human glioma cell line (U251) in vitro. Materials and Methods: U251 cells were transfected with FGFR1-mCherry and treated with fluorescently labeled FGF2. Down-regulation of SPRY2 was achieved by treatment with specific siRNAs. Immunostaining and fluorescence microscopy as well as Western blotting were performed to analyze trafficking and the activation of downstream signaling cascades. Moreover, molecular models of SPRY2 and its interaction partners were generated with PyMol. Results: Down-regulation of SPRY2 resulted in increased localization of FGFR1 and FGF2 in the early endosomal and recycling compartments, but reduced colocalization with the degradation compartment (lysosomes). Furthermore, SPRY2 siRNAs led to enhanced cell proliferation of the FGFR1 overexpressing cells, which was accompanied by a significant increase of AKT activity. Conclusions: Our results demonstrate that SPRY2 acts as stimulator of PI3K/AKT signaling in FGFR1 expressing human glioma U251 cells via modulation of FGF receptor trafficking in vitro. To determine its role as putative tumor suppressor or oncogene in glioblastoma xenograft transplantation experiments will be necessary.

RAMAN SPECTROSCOPY FOR INTRAOPERATIVE DIAGNOSIS OF BRAIN TUMORS

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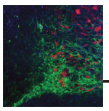
Raman spectroscopy reveals the molecular composition of tissue. It is suitable for in situ diagnosis of glioma and provides prognostic information, for instance about the IDH1 genotype. Therefore, Raman spectroscopy is regarded as promising tool to obtain an integrated diagnosis of brain tumors in situ. However, studies have been performed on a small number of patients or on croysections until now. Here, we investigated fresh tissue biopsies to approximate the intraoperative setting for evaluation of the potential of the technique. 208 fresh biopsies were obtained during brain tumor surgery and directly subjected to Raman spectroscopy. Samples from surgeries for treatment of pharmaco resistant epilepsy served as non-tumor control tissue. Raman bands related to hemoglobin proved to be valuable markers for the detection of blood contamina-



tion. All tumor types showed a significant reduction in the spectral range 1050-1150 cm-1 compared to non-tumor brain tissue according to the Fisher's coefficient. Bands related to lipids (1297, 2850, 3011 cm-1) were strongly reduced in glioblastoma. Furthermore, the band at 1660 cm-1 (assigned to protein) was increased. These spectral changes were less pronounced in recurrent glioblastoma or low grade glioma. The band of cholesterol at 700 cm-1 was largely missing in brain metastases and bands assigned to collagen were more intense. The Raman spectra of fresh biopsies measured in the operating room showed tumor-specific spectral patterns, opening the possibility of in situ tissue classification with high sensitivity and specificity. Perspectively, this will assist neuropathologists and neurosurgeons to obtain early - intraoperative - integrated diagnosis and to consecutively apply personalized local therapies.

COMBINED ENDOSCOPIC AND RADIOSURGICAL TREATMENT OF ESTHESIOBEUROBLASTOMA

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Microsurgical techniques have considerably improved the results from surgical treatment of esthesioneuroblastoma (olfactory neuroblastoma). Nevertheless, these rare tumours of the frontal skull base are still associated with high rates of tumour recurrence and mortality, thus remaining a challenge even for experienced surgeons. A novel therapeutic approach that combines endoscopic sinus surgery and radiosurgery (Gamma Knife) is presented here. Thirteen patients (8 males, 5 females) aged between 27 and 75 years (median 38 years) were treated. Following paranasal and nasal endoscopic sinus surgery marginal irradiation doses ranging from 15 to 34 Gy were applied radiosurgically involving 1 to 7 isocentres. At present, the median follow-up period is 64 months (range 4-118 months). There was no mortality. Tumour control was achieved in the treated area. Four patients underwent a second radiosurgical procedure. Two patients had to undergo additional craniotomy because of extensive neoplastic infiltration, one of them developed postoperative liquorrhea. In another case the clinical course was complicated by a bilateral frontal sinusitis. All patients complained of nasal discharge and crusts. However, a preoperative Karnovsky Index ranging from 80% to 100% remained stable in all patients whereas an improvement was observed in two patients. Based on the favourable results observed so far, the combination of endoscopic sinus surgery and radiosurgery can be considered a promising new option for the treatment of esthesioneuroblastoma that merits further investigation.

GLIOMA-ASSOCIATED MESENCHYMAL STEM CELLS PROMOTE TUMOUR CELL INVASION

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The glioblastoma (GBM) microenvironment contains a range of cell-types, including mesenchymal stem cells (MSCs), modulating tumour progression. MSC are tissue-resident stem cells, which are activated e.g. by injuries and can coordinate wound healing processes. It is established that peripheral MSC home to GBM via the bloodstream and there is a debate if MSC mediate pro- or anti-tumourigenic effects. Here, we investigated the parameters that control tumour –repressive or -supportive functions of MSC and found that exposure of MSC to blood serum is a determining factor. Cultivated MSC in a serum-containing environment reduce the cell viability of a range of human primary stem like GBM cells (GSC), while MSC in serum-containing medium induce the opposite effect. Next we investigated which condition is pathologically more appropriate and performed different orthotopic implantation experiments with GSC and MSC in mice. We observed that MSCs co-injected with GBM are rapidly lost from the tumour mass, but that MSC and GBM cells occur approximately at a 1:1 ratio in small intraparenchymal tumour satellites. Similar results were obtained when applying MSCs to established tumours. We concluded that MSC neighbouring with GBM cells are located in tissue areas that are still largely intact, with no vascular leakiness and no exposure to blood-serum. Under such conditions (in vitro) MSCs shed extracellular vesicles (EV), which are incorporated by GBM cells, promote morphological changes and induce invasiveness. Gene expression analysis of MSC, EV and GBM-cells (challenged with MSC-derived EVs or controls) revealed that MSC induce the expression of the ephrin-ligand EFNA3 in GBM cells. Genetic manipulation of EFNA3 in MSC and GBM indicated that this ligand is necessary for the MSC-induced invasion of GBM cells. Altogether, we provide an explanation for the diverse effects of MSC in GBM and indicate a treatment target to blunt MSC-induced invasion.

THE DUAL EFFECT OF CNF1 ON GLIOMA: ANTI-NEOPLASTIC AGENT AND GUARDIAN OF BRAIN FUNCTION.

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Gliomas are malignant primary tumors of the central nervous system that arise from astrocytes, oligodendrocytes and their precursors. Glioblastoma (GBM) is the most aggressive type of glioma with a median survival expectancy of 15-18 months since the first diagnosis. The standard of care in GBM patients consists in the surgical resection of tumor mass followed by cycles of radiotherapy and chemotherapy. But this combined intervention protocol is only partly effective; thus, there is an urgent need to find innovative and efficient approaches for the treatment of GBM patients. In particular, these novel strategies should aim not only at targeting glioma growth but also at preventing functional deterioration of spared brain networks. We assessed the effectiveness of an anti-glioma therapy based on the administration of Cytotoxic Necrotizing Factor 1 (CNF1), a bacterial protein toxin leading to the long-lasting activation of intracellular Rho GTPases. Remarkably, we showed that CNF1 (i) leads to multinucleation, senescence and death of murine and human glioma cells in vitro, (ii) increases the survival of glioma-bearing mice, (iii) spares neuronal responses and architecture in the tissue surrounding glioma mass. To better characterize the translational value of this therapeutic strategy, we also treated glioma-bearing mice with CNF1 at the symptomatic stage and we evaluated whether Rho GTPase activation was effective in halting tumor growth progression and in protecting neuronal cells from functional deficits. We found that CNF1 treatment within a clinically relevant time window is successful in stabilizing/reducing glioma volume and the associated physiological deficits. In contrast, TMZ treatment at symptomatic stage is ineffective in preserving motor performance, indicating that TMZ-based therapy does not contribute to preserve animals' functional capabilities as CNF1 does. Altogether, these data demonstrate that CNF1 could be considered a novel promising strategy for the development of a more effective glioma therapy.

UNCOVERING PREDICTIVE GENETIC- AND METABOLIC-MARKERS FOR GBM THERAPY

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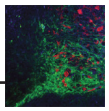

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Glioblastoma (GBM) is characterized by large inter-patient heterogeneity, which is caused by different genetic mutations generating diverse biological phenotypes. As a practical approach for more individualized therapies GBM can be categorized into distinct genetic or metabolic subsets. The plant-derived vanilloid cannabidiol (CBD) is advanced to phase II clinical trials, but predictive markers for CBD-therapy are missing. We engineered more than 30 different transgenic mouse glioma lines and generated 24 patient-derived GBM-cultures with identified genetic / genomic aberrations to define molecular markers correlating with CBD-induced cytotoxicity in GBM cells. We found that a simple, combinatorial set of genetic driver mutations can be used to identify CBD-responsive and unresponsive subsets. Furthermore, we show that the p53-status in CBD-sensitive GBM determines the cell-death pathway. NMR-measurements and cell-biological analysis reveal that CBD induces large alterations in lipid-, amino acid-, and energy metabolism, inflammatory response as well as antioxidant defence in CBD-sensitive tumours. Importantly, we found that metabolic patterns, like the level of reactive oxygen species (ROS) in GBM, provide a robust measure to segregate GBM into CBD-therapy responders and non-responders before application of the drug. We show in a preclinical setting that GBM biopsies can be identified as CBD-sensitive (or insensitive) tumours after staining with a ROS indicator dye. In the next step we will validate additional metabolic markers with predictive capacity for CBD-treatment and apply molecular imaging techniques (MRI and PET) to visualize these markers. This approach will then provide the advantage not only to resolve inter-patient but also intra-tumoural heterogeneity and may guide therapeutic decisions.

ROLE OF AURORA KINASE A IN THE THERAPEUTIC RESISTANCE OF GLIOBLASTOMA ASSOCIATED WITH THE PRODUCTION OF CXCL12 IN THE SUBVENTRICULAR ZONES

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We previously demonstrated that glioblastoma-initiating cells, highly tumorigenic subpopulations of glioblastoma (GBM) cells, are able to escape the tumor mass, to migrate along the corpus callosum and to specifically invade the subventricular zones (SVZ) in response to the local production of the CXCL12 chemokine. SVZ are stem cells niches crucial for adult neurogenesis, which are particularly propitious for GBM development and therapeutic resistance. In this work, we study the role of the mitotic Aurora A (AurA) kinase in the therapeutic resistance associated with SVZ invasion. We showed that AurA is phosphorylated in human GBM cells after stimulation with the CXCL12 chemokine. Treatment with Alisertib, a specific P-AurA inhibitor, blocks the chemotaxis of GBM cells induced by CXCL12 using in vitro migration bio-assays. Similarly, SVZ invasion was significantly reduced in a xenograft mouse model of GBM. Finally, we showed that AurA also reinforces the radio-resistance conferred by CXCL12, itself associated with a mesenchymal activation of GBM cells. These data showed that AurA contributes to the escape of GBM cells from the TM to the SVZ and to their locally acquired radio-resistance, both conferred by the production of CXCL12 in the SVZ. We proposed that the mesenchymal pattern induced by AurA underlies these CXCL12 specific responses in GBM cells.

THE CANCER STEM CELL FACTOR ALDEHYDE DEHYDROGENASE 1A3 (ALDH1A3) IS REGULATED BY AUTOPHAGY IN HUMAN GLIOBLASTOMA CELLS

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Aldehyde dehydrogenases (ALDH) have been identified as markers for the cancer stem cell phenotype in different tumor types. It has been shown that ALDH1A3 is expressed in glioblastoma and that its expression correlates with a worse prognosis. In the present study ALDH1A3 expression was associated with resistance against TMZ treatment and sensitivity could be re-established in ALDH1A3 knockout cells. TMZ treatment at high concentrations diminished ALDH1A3 protein and this downregulation leads to re-sensitization of the tumor cells to chemotherapy. ALDH1A3 was post-transcriptionally regulated since mRNA levels were not affected by TMZ treatment. With increasing concentrations of TMZ, autophagy was up-regulated, and we found evidence for a physical interaction between ALDH1A3 and p62, an important adaptor protein in autophagosomes indicating that ALDH1A3 protein is downregulated by autophagy. So far, the results of the exact role of autophagy in tumor development and tumor growth are inconsistent. Our data indicate that the cancer stem cell factor ALDH1A3, that is directly involved in therapy resistance of glioblastoma, is regulated by autophagy during chemotherapy.

PROGRAMMED CELL DEATH 10 IN GLIOBLASTOMA

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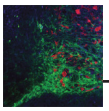
Objective Neovascularization and peritumoral edema are hallmarks of glioblastoma (GBM). Programmed cell death 10 (PDCD10) plays a pivotal role in regulating apoptosis, neoangiogenesis and vessel permeability and is implicated in certain tumor signaling pathways. However, little is known about PDCD10 in GBM. We aimed to investigate the expression pattern of PDCD10 and to identify the association of its expression with some molecular and clinical parameters in human GBM. We also attempted to study the role of PDCD10 in tumor cell behaviours and in tumor progression. Methods mRNA and protein expression of PDCD10 were examined respectively by real-time RT-PCR and Western blotting in GBM (n=27), astrocytoma grade II (n=13) and control (n=11). The association of PDCD10 expression with brain edema and microvascular density (MVD) were analysed based on pre-operative MRI and after laminin immunostaining. The role of PDCD10 on GBM cell proliferation, adhesion, migration and invasion, on tumor growth and tumor angiogenesis were studied after shRNA knockdown of PDCD10 in vitro and in a mouse model of GBM. The underlying mechanism was explored by a protein array. Results mRNA and protein levels of PDCD10 were significantly downregulated in GBM, concomitantly accompanied by the activation of Akt. PDCD10 immunoreactivity was absent in proliferating tumor cells, endothelial cells and GFAP-positive cells, but exclusively present in the hypoxic pseudopalisading cells which underwent apoptosis. Moreover, loss of PDCD10 was associated with a higher MVD and a more severe peritumoral edema but not with MGMT promoter methylation in GBM. Knockdown of endo thelial PDCD10 markedly activated GBM cells and promoted tumor growth accompanied with a massive neo-angiogenesis. Protein array revealed a significant increased release of 22 of 55 detected angiogenic factors from PDCD10-knockdown endothelial cells. Conclusion



We report for the first time that PDCD10 expression is downregulated in GBM, which is associated with the activation of survival signalling and with hyperangiogenesis and peritumoral edema in human GBM. Loss of endothelial PDCD10 can activate tumor cells and stimulates tumor growth through the upregulation of multiple angiogenic factors. These finding suggest PDCD10 as a tumor suppressor-like function and PDCD10 could be a potential novel target for anti-angiogenesis therapy of GBM.

INHIBITION OF CXCR2/CXCL2 SIGNALING PATHWAY IN GLIOBLASTOMA MULTIFORME AS A THERAPEUTIC APPROACH

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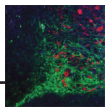
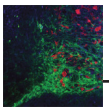
INTRODUCTION: Signaling via CXCR2 and its ligands CXCL2 and CXCL8 is a crucial part of angiogenesis in glioblastoma. The aim of our study was to block this signaling pathway via CXCR2-Antagonist SB225002 as a new therapeutic approach to slow down tumor growth. METHODS: Glioma cells were implanted intracranially. Mini osmotic pumps were inserted in the left ventricle to deliver either SB225002 or placebo dilution. The 14 or 7 day period of treatment was initiated on the day of tumor cell implantation (n=6-7 per group) or after 14 days of tumor growth (n=6-7 per group). Tumor volume was measured using MRI before starting the treatment and at the end of the treatment period. Immunofluorescence stainings for vascularization and phenotype of microglia cells were performed. RESULTS: Treatment with SB225002 led to significantly reduced tumor volume of 51 % after initial CXCR2-blocking and of 47 % after blocking the receptor during exponential tumor growth period. The immunofluorescence stainings showed significantly reduced number of microglia cells and a diminished expression of the CXCR2 receptor in the group with initial receptor blocking. For the late therapy group, accumulation of microglia cells was slightly reduced. Vascularization in both groups was not altered. CONCLUSION: The CXCR2 antagonist SB225002 significantly reduced glioblastoma growth during tumor initiation and growth phase and represents a promising therapeutic agent. Further investigations are needed to clarify underlying molecular pathways and to optimize the treatment.





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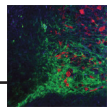

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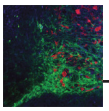

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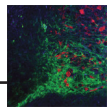

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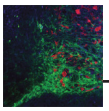

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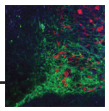

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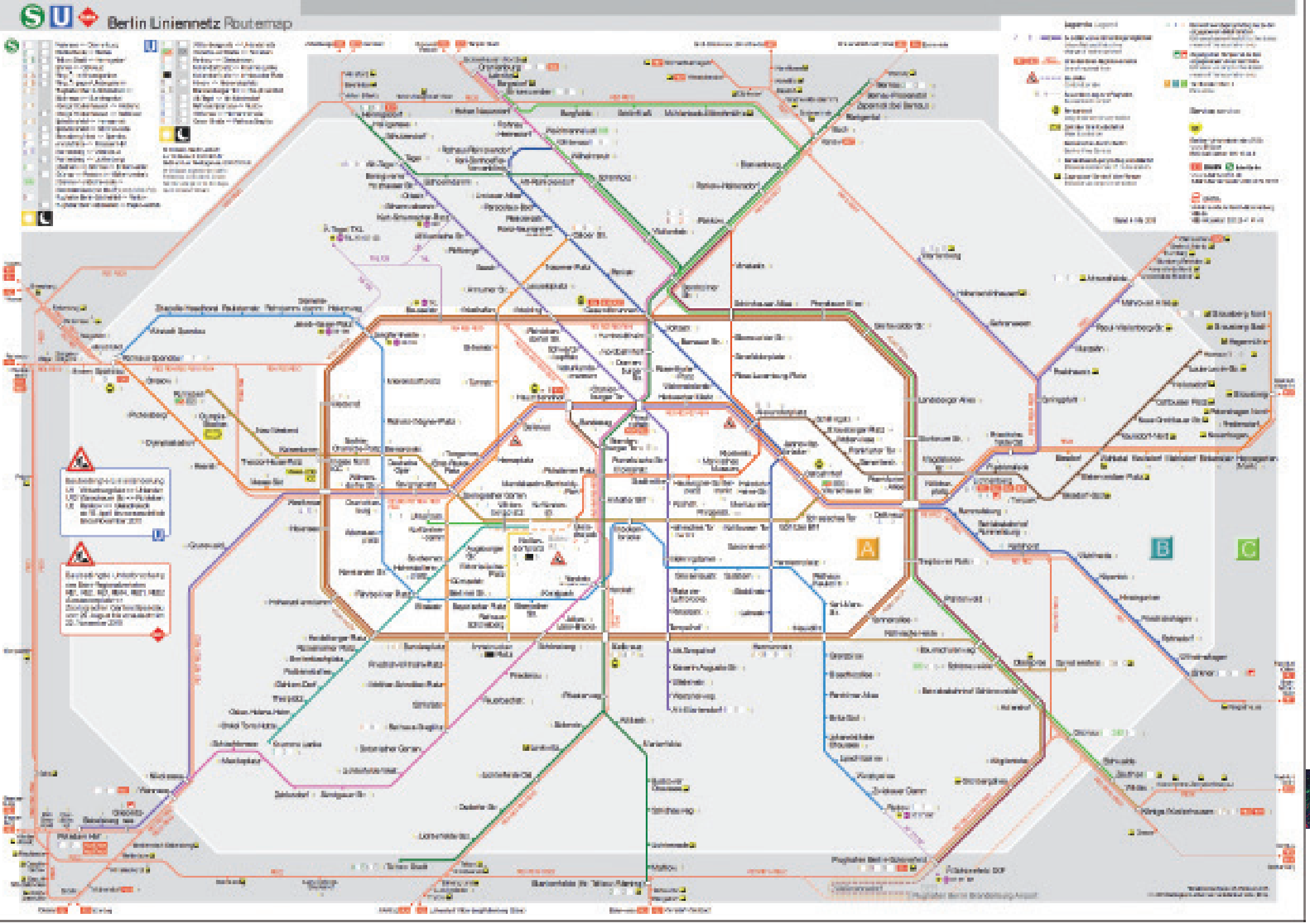
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