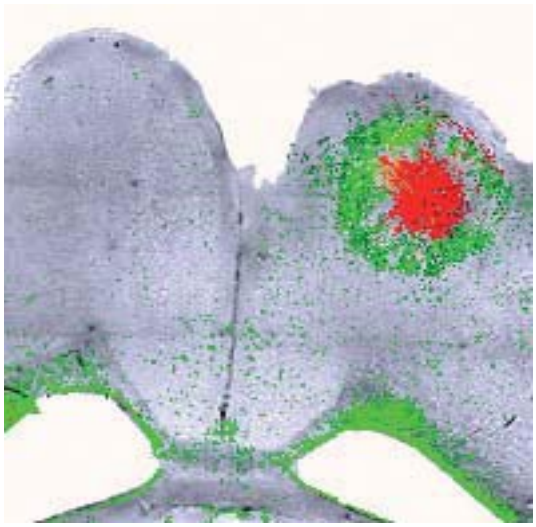
Brain Tumor 2006



Program and Abstracts

(Orals and Posters)

December 7 / 8, 2006

Campus Berlin-Buch
Max Delbrück Communications Center (MDC.C)
Robert-Rössle-Str. 10
D13125 Berlin

HELIOS Kliniken - MDC

MDC Berlin-Buch Max Delbrück Center for Molecular Medicine

Scientific Program

Thursday, December 7, 2006

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

14.05 - 15.45 **Session I**
Chair: Walter Birchmeier

14.05 – 14.45 **Steven Goldman** (Rochester, USA)
Differential gene expression by human CNS tumor stem cells

14.45 – 15.25 **Otmar Wiestler** (Heidelberg)
Cancer Stem Cells: a new paradigm in oncology research

15.25 - 15.45 **Virginie Clément** (Geneva, Switzerland)
SONIC HEDGEHOG-GLI signaling regulates glioma growth and the behavior of glioma cancer stem cells

15.45 – 16.15 **Poster Session and Coffee Break**

16.15 – 17.55 **Session II**
Chair: Frauke Zipp

16.15 – 16.55 **Michael Weller** (Tübingen)
Novel mediators of glioblastoma-associated immune suppression

16.55 - 17.35 **Bozena Kaminska** (Warzaw, Poland)
Counteracting microglia-glioma interactions as novel target for therapy of malignant glioma

17.35 – 17.55 **Michael Synowitz** (Berlin)
A1 adenosine receptors in microglia control glioblastoma – host interaction

17.55 – 18.15 **Coffee Break and Poster Session**

18.15 – 19.55 **Session III**
Chair: Jürgen Kiwit

18.15 – 18.55 **Peter Vajkoczy** (Mannheim)
Angiogenesis of malignant brain tumors

18.55 – 19.35 **Jörg-Christian Tonn** (Munic)
The VEGFR-3 system – a major mediator in glioma angiogenesis ?

19.35 – 19.55 **Karl H. Plate** (Frankfurt)
Bone-marrow derived cells contribute significantly to glioma angiogenesis and growth

20.00 - 20.30 **Bus Transfer to Virchow-Ruine**

20.30 **Reception in the Virchow Ruine / Charité**

Friday, December 8, 2006

9.00 - 10.40 **Session IV**
Chair: Helmut Kettenmann

9.00 - 9.40 **Mark Noble** (Rochester, USA)
The normal brain as susceptible bystander of cancer treatments: In vitro and in vivo studies, and novel pathways of action of therapeutic agents.

9.40 - 10.20 **Manuel Guzman** (Madrid, Spain)
Cannabinoids as potential antitumoral agents for gliomas

10.20 – 10.40 **Rolf Mentlein** (Kiel)
The chemokine receptor cxcr6/ bonzo defines a migratory subset of glial cells in astrocytomas

10.40 – 11.10 **Poster Session and Coffee Break**

11.10 - 12.10 **Session V**
Chair: Gerd Kempermann

11.10 – 11.50 **Manfred Westphal** (Hamburg)
Translation of glioma biology into glioma stem cell biology

11.50 – 12.10 **Rainer Glass** (Berlin)
Cell-intrinsic limitations of the neural precursor cell response to gliomas

12.10 - 13.00 **Lunch**

13.00 – 14.40 **Session VI**
Chair: Andreas von Deimling

13.00 – 13.20 **Nicolai Savaskan** (Amsterdam, Netherlands)
A role for the micronutrient selenium in glioma cell growth and brain invasion

13.20 – 13.40 **Barbara Böck** (Heidelberg)
The PEA15/PED protein protects glioblastoma cells from glucose depriviation-induced apoptosis via the ERK/MAP kinase pathway

13.40 – 14.00 **Donat Kögel** (Frankfurt)
Proteasome inhibitors efficiently reactivate TRAIL-induced apoptosis in malignant glioma

14.00 – 14.40 **Peter Lichter** (Heidelberg)
Elucidation of pathomechanisms in human brain tumors by molecular profiling

14.40 **Departure**

List of Orals

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Böck, B.; Eckert, A.; Herold-Mende, C.; Walczak, H.; Wiestler, O.; Roth, W.
German Cancer Research Center, Molecular Neuro-Oncology, Im Neuenheimer Feld 280, 62120 Heidelberg, eMail: b.boeck@dkfz-heidlberg.de

SONIC HEDGEHOG-GLI SIGNALING REGULATES GLIOMA GROWTH AND THE BEHAVIOR OF GLIOMA CANCER STEM CELLS
Clément, V.; Sánchez, P.; Radovanovic, I.; de Tribolet, N. ;Ruiz i Altaba, A.
University of Geneva Medical School, Genetic Medcine and Development, 1 rue michel servet, 1211 Geneva Swizterland, eMail: Virginie.Clement@medecine.unige.ch

CELL-INTRINSIC LIMITATIONS OF THE NEURAL PRECURSOR CELL RESPONSE TO GLIOMAS
Glass, R.
MCD, Cellular Neuroscience, Robert Rössle Str 10, 13125 Berlin, eMail: rainer.glass@mdc-berlin.de

PROTEASOME INHIBITORS EFFICIENTLY REACTIVATE TRAIL-INDUCED APOPTOSIS IN MALIGNANT GLIOMA
Hetschko, H.; Weissenberger, J.; Kögel, D.
Frankfurt University Clinics, Experimental Neurosurgery, Theodor-Stern-Kai 7, 60590 Frankfurt, eMail: koegel@em.uni-frankfurt.de

THE CHEMOKINE RECEPTOR CXCR6 / BONZO DEFINES A MIGRATORY SUBSET OF GLIAL CELLS IN ASTROCYTOMAS
Menltein, R.; Hattermann, K.; Ludwig,A.; Kruse, M.-L.; Held-Feindt, J.
University of Kiel, Anatomy, Olshausenstr. 40, 24098 Kiel, eMail: rment@anat.uni-kiel.de

BONE-MARROW DERIVED CELLS CONTRIBUTE SIGNIFICANTLY TO GLIOMA ANGIOGENESIS AND GROWTH
Marcia R. Machein (1), Mark Kerber (1), Christine Mayer (1), Anke Wickersheim (1), Fabian Kiessling (2), Manfred Jungold (2), Mathias Heil (3), Yvonne Reiss (4), Sabine Raab (4) and Karl H. Plate (4)(
Klinikum J.-W. Goethe-Universität Frankfurt, Neurologisches Institut, Deutschordenstr. 46, 60528 Frankfurt, eMail: karl-heinz.plate@kgu.de

A ROLE FOR THE MICRONUTRIENT SELENIUM IN GLIOMA CELL GROWTH AND BRAIN INVASION
Savaskan, N.E.; Hahnen, E.; Ganslandt, O.; Buchfelder, M.; Nimsky, C.; Eyüpoglu, I.Y.
The Netherlands Cancer Institute, Division of Cellular Biochemistry, Plesmanlaan 121, 1066CX Amsterdam The Netherlands, eMail: n.savaskan@nki.nl

A1 ADENOSINE RECEPTORS IN MICROGLIA CONTROL GLIOBLASTOMA – HOST INTERACTION
Synowitz, M.
Helios Hospital, Department of Neurosurgery, Hobrechtsfelder Chaussee 96, 13125 Berlin, eMail: MSynowitz@berlin.helios-kliniken.de

Abstracts of Oral Presentations

THE PEA15/PED PROTEIN PROTECTS GLIOBLASTOMA CELLS FROM GLUCOSE DEPRIVATION-INDUCED APOPTOSIS VIA THE ERK/MAP KINASE PATHWAY

Barbara Böck, Anika Eckert, Christel Herold-Mende, Henning Walczak, Otmar Wiestler, Wilfried Roth
Molecular Neuro-Oncology, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg

PEA15/PED (Phosphoprotein enriched in astrocytes 15 kD/ Phosphoprotein enriched in diabetes) is a death effector domain protein involved in the regulation of apoptosis, insulin resistance, and MAP kinase signaling. Since PEA15 is highly expressed in cells of glial origin, we studied the role of PEA15 in malignant glioma. Phosphorylation of PEA15 at S116 is found *in vivo* in perinecrotic areas in glioblastomas and *in vitro* after glucose deprivation of glioblastoma cells. Phosphorylation of PEA15 upon glucose withdrawal is blocked by BIS-VIII, a PKC inhibitor, or KN-93, a CaM kinase inhibitor. Overexpression of PEA15 induces marked resistance against glucose deprivation-induced apoptosis, whereas downregulation of endogenous PEA15 by transfection of specific siRNA oligonucleotides results in sensitization to glucose withdrawal-mediated cell death. The anti-apoptotic activity of PEA15 under low glucose conditions depends on its phosphorylation, since the protective effect is abrogated by inhibitors of PKC and CaM kinase. Moreover a non-phosphorylatable PEA15 mutant (S104A/S116A) does not confer resistance to glucose withdrawal. PEA15 regulates the level of phosphorylated ERK1/2 in glioblastoma cells. Since treatment with an ERK1/2 inhibitor abolishes the PEA15-mediated resistance, we conclude that PEA15-dependent protection from glucose deprivation-induced apoptosis requires ERK1/2 signaling. Taken together, our findings suggest that phosphorylated PEA15 renders glioma cells resistant to glucose deprivation in poor microenvironments, such as in perinecrotic areas of glioblastoma multiforme. Increased PEA15 expression might also contribute to cellular resistance of malignant glioma to chemo- and radiotherapy.

SONIC HEDGEHOG-GLI SIGNALING REGULATES GLIOMA GROWTH AND THE BEHAVIOR OF GLIOMA CANCER STEM CELLS.

V. Clément, P. Sánchez, I. Radovanovic, N. de Tribolet and A. Ruiz i Altaba
Department of Genetic Medicine and Development, University of Geneva Medical School and Cantonal Hospital, Geneva, Switzerland.

Sonic hedgehog (Shh) signaling controls several aspects of ontogeny, orchestrating congruent growth and patterning. During brain development, Shh first regulates early ventral patterning while later on it is critical for the regulation of precursor proliferation in the dorsal brain, namely in the neocortex, tectum and cerebellum (Dahmane and Ruiz i Altaba, 1999; Dahmane et al., 2001). We have previously shown that Shh controls not only the behavior of cells with stem cell properties in the mouse embryonic neocortex (Palma et al., 2004), but also that Shh is required for cell proliferation in the mouse forebrain's subventricular zone (SVZ) stem cell niche, suggesting a critical and conserved role of Shh signaling in the regulation of stem cell lineages in the adult mammalian brain (Palma et al., 2005). In parallel, we have shown that misregulation of SHH-GLI function can be a causative event in tumorigenesis (Dahmane et., 1997; Dahmane et al., 2001) and that its sustained activity is required for the continued growth of tumors from different tissues including the brain (Dahmane et al, 2001). Specifically, we showed that a number of human brain tumors, including gliomas and medulloblastomas, require SHH signaling (Dahmane et al, 2001). We now provide evidence that SHH-GLI signaling controls the behavior of both glioma cancer stem cells, and defines their two sinequanon properties: self-renewal and tumorigenicity. Interference with SHH signaling inhibits human glioma xenograft growth in mice, confirming that the growth of the tumor bulk depends on an active SHH-GLI signaling. Most importantly, SHH signaling controls the number of self-renewing glioma cancer stem cells and regulates a battery of genes implicated in self-renewal. These results reveal a striking dependence of glioma cancer stem cells and normal brain stem cells on SHH-GLI signaling that may be extended to other solid human cancers.

PROTEASOME INHIBITORS EFFICIENTLY REACTIVATE TRAIL-INDUCED APOPTOSIS IN MALIGNANT GLIOMA

H. Hetschko, J. Weissenberger, D. Kögel
Experimentelle Neurochirurgie, Zentrum für Neurologie und Neurochirurgie, Johann Wolfgang Goethe-Universität Frankfurt

Glioblastoma multiforme (GBM) is characterized by potent resistance against apoptosis and antineoplastic treatment. The purpose of this study was to elucidate dysfunctions in apoptotic signaling cascades and to evaluate the efficiency of novel therapeutic approaches to reactivate apoptosis in GBM. Here we investigated the sensitivity of a panel of six human GBM cell lines (established GBM cell lines U87, U251, U343 and U373, and two additional cell lines [MZ-54, MZ-18] derived from patients with grade IV astrocytomas) to apoptosis induced by the death receptor ligand TRAIL (250 ng/ml), TRAIL in combination with gamma-irradiation (20 Gy), TRAIL in combination with proteasome inhibitors (2,5 µM MG132; 50 nM epoxomicin), or Bcl-2/Bcl-xL inhibitors (30 µM HA14-1, 30 µM BH3-I2´). In-depth analysis of six of the GBM cell lines revealed drastic differences in their sensitivity to these treatments, with two of the six cell lines revealing no significant induction of cell death in response to TRAIL alone. Interestingly, the combinatory treatments with TRAIL revealed that apoptosis could be efficiently reactivated in the TRAIL-resistant cell lines with the Bcl-2/Bcl-xL inhibitors BH3-I2´ and HA14-1, and even more potently with the proteasome inhibitors MG132 and epoxomicin. Further analyses employing RNA interference techniques and gene expression arrays carried out to identify the molecular signaling pathways leading to reactivation of TRAIL sensitivity induced by proteasome inhibitors focused on the roles of the transcription factors GADD153/CHOP, c-Jun and NF-kB and downstream induction of the death receptor DR5. Novel therapeutic approaches with TRAIL and angonistic TRAIL receptor antibodies in combination with proteasome inhibitors might be a promising therapy approach to efficiently reactivate apoptosis in therapy-resistant GBMs in the future.

THE CHEMOKINE RECEPTOR CXCR6 / BONZO DEFINES A MIGRATORY SUBSET OF GLIAL CELLS IN ASTROCYTOMAS

Rolf Mentlein,[1] Kirsten Hattermann,[1] Andreas Ludwig,[2] Marie-Luise Kruse,[4] Janka Held-Feindt[3]
Departments of [1]Anatomy and [2]Biochemistry, University of Kiel, Kiel, Germany. Departments of [3]Neurosurgery and [4]General Internal Medicine, Universitätsklinikum Schleswig-Holstein Campus Kiel, Kiel, Germany

Chemokines and their receptors play a decisive role in tumor progression and metastasis. Here, we describe the expression of the CXCL16-CXCR6-system in human astroglial brain tumors (astrocytomas, gliomas). The transmembrane chemokine CXCL16 is overexpressed on the mRNA and protein level in all astrocytomas investigated as well as in all glioma cell lines. Its receptor CXCR6 is highly expressed in most, but not all astroglial tumors, but undectable in normal adult human brain. As evidenced by confocal laser microscopy, CXCR6-expressing cells represent a subpopulation of proliferating cells that are partly positive for the astroglial marker glial fibrillary acidic protein GFAP and mostly positive for the stem cell marker Nestin, but negative for the chemokine receptor CXCR4. Conventionally cultivated glioma cell lines are negative for CXCR6, but immature astroglial cells are CXCR6-positive. In vitro, stimulation of CXCR6-positive astroglial cells by soluble CXCL16 activates mainly the PI3-kinase / Akt

pathway resulting in the activation of the transcription factor AP-1, but not of NFêB. As consequence, soluble CXCL16 upregulates its own receptor CXCR6, induces cell proliferation and cell migration in wound-healing and spheroid confrontation assays that can be inhibited by the PI3-kinase inhibitor Wortmannin, or the later by sheddase inhibitors and CXCL16-antibodies. Thus, CXCL16 contributes to tumor growth by recruitment of CXCR6-positive tumor/glial precursor cells and by paracrine proliferating effects. CXCR6 expression in glial tumors reveals their high heterogeneity and supports a general concept on the role of chemokine / receptors in attracting cancer stem cells.

Supported by an intramural grant of the Universitätsklinikum Schleswig-Holstein Campus Kiel (JH-F & RM) “Stem cells in brain tumors” and in part by the State of Schleswig-Holstein grant “Molecular Imaging in the North MOIN” (RM).

A ROLE FOR THE MICRONUTRIENT SELENIUM IN GLIOMA CELL GROWTH AND BRAIN INVASION

Nicolai E. Savaskan[*1], Eric Hahnen[2], Oliver Ganslandt[3], Michael Buchfelder[3], Christopher Nimsky[3] & Ilker Y. Eyüpoglu[3]

[1]Division of Cellular Biochemistry, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [2]Institute of Genetics and Center for Molecular Genetics Cologne (CMMC), University of Cologne, Germany; [3]Department of Neurosurgery, University of Erlangen-Nuremberg, Germany.

Malignant brain tumors grow destructive and invasive inducing thereby massive cell death in adjacent brain parenchyma. However, a major concern in brain tumor therapy is the low efficacy of current treatment protocols. Also, patients with primary brain tumors show complex alterations in cerebrospinal fluid components such as reduced levels of the micronutrient selenium. Since selenium is essential for proper neural functioning, we tested this trace element in different glioma cell lines and in an organotypic glioma invasion model (OGIM). Selenium was highly toxic and induced apoptosis in glioma cells, whereas neurons and non-transformed cells were not affected. Treatment with selenium in form of selenite inhibited glioma-originated glutamate secretion and protected from neuronal cell death and microglial activation. Hence, selenite was highly effective in preventing glioma expansion and peritumoral cell death. Molecular analysis further revealed that selenium treatment in glioma cells leads to rapid phosphoinositide phosphatidylinositol bisphosphate (PIP_2) depletion. Thus, recovering PIP_2 levels by expression of the PIP_2-generating enzyme PIP5-kinase 1a could reduce selenium-induced glioma cell death. Furthermore, selenium induced cell death was independently of and prior caspase activation. These data support the notion that the micronutrient selenium induces highly specific apoptosis in glioma cells in a PIP_2 dependent manner while protecting neurons and reducing inflammation. Thus, the selenium-mediated killing strategy of gliomas may be useful in treating gliomas and in protecting brain cells from apoptosis.

This work is supported by the Deutsche Forschungsgemeinschaft (DFG, SA1041/4-2) and the Human Frontier Science Program (HFSP).

BONE-MARROW DERIVED CELLS CONTRIBUTE SIGNIFICANTLY TO GLIOMA ANGIOGENESIS AND GROWTH

Marcia R. Machein (1), Mark Kerber (1), Christine Mayer (1), Anke Wickersheim (1), Fabian Kiessling (2), Manfred Jungold (2), Mathias Heil (3), Yvonne Reiss (4), Sabine Raab (4) and Karl H. Plate (4)(1)

Department of Neurosurgery, Freiburg University Medical School, Freiburg, Germany(2) Department of Experimental Radiology and Physics, DKFZ, Heidelberg, Germany(3) Max Planck Heart and Lung Institute, Bad Nauheim, Germany(4) Institute of Neurology (Edinger Institute), Frankfurt University Medical School, Frankfurt, Germany

In glioblastoma, the most common and most malignant neuroectodermal tumor, angiogenesis is primarily driven by hypoxia which leads to upregulation of hypoxia-inducible factor-1 (HIF). HIF-1 transcriptionally activates more than 100 genes, including the prominent tumor angiogenesis factor vascular endothelial growth factor (VEGF). Until recently it was believed that VEGF produced by hypoxic tumor cells would the major (if not only) stimulus for tumor angiogenesis in glioblastoma. However, monocytes/macrophages express VEGFR-1 and are also major producers of VEGF. It has been shown recently that the production of VEGF by monocytes/macrophages is dependent on VEGFR-1 signalling (Murakami M et al, Blood 108, 2006). Since glioblastomas contain a considerable amount (up to 50% of the total tumor mass) of macrophages we tested the relative contibution of monocyte/macrophage derived VEGF versus tumor derived VEGF in a murine glioma model. Bone marrow (BM) from transgenic mice with a signalling deficient VEGFR-1 (VEGFR-1 TK-/-eGFP) was tranplanted into sublethally irradiated wild type mice. After bone marrow reconstitution the resulting chimeric mice received intracerebral inocculation of GL261 glioma cells. Intracerebral tumor growth analyzed by both MRI and histology showed a significant slower tumor growth in VEGFR-1 signalling deficient BM-chimeras compared to wild-type controls. Histological analysis revealed a defect in tumor vascularization as a contibuting cause of diminished tumor growth. In order to rescue tumor angiogenesis GL261 cells stably transfected with murine VEGF were transplanted into VEGFR-1 signalling deficient BM-chimeras. GL261 gliomas that overexpressed VEGF showed a similar growth rate, blood volume and vascular permeabiliy in VEGFR-1 signalling deficient and wild-type BM-chimeras. Our findings suggest an unexpected major contribution of BM-derived cells to tumor angiogenesis and identify monocytes/macrophages as a novel target to anti-angiogenic tumor therapies.

MICROGLIA MEDIATED A1 ADENOSINE RECEPTOR ACTIVITY MODULATES THE GROWTH OF EXPERIMENTAL GLIOBLASTOMA CELLS

Michael Synowitz[1,2], Rainer Glass[2], Katrin Faerber[2], Golo Kronenberg[3], Juergen Schnermann[4,] Helmut Kettenmann[2]

[1]Department of Neurosurgery, Helios Hospital Berlin, Hobrechtsfelder Chaussee 96, 13125 Berlin, Germany.
[2]Cellular Neuroscience Group, Max Delbrück Center for Molecular Medicine (MDC), Robert Rössle Str. 10, 13092 Berlin, Germany.
[3]Department of Psychiatry, Free University, Charité – CBF, Eschenallee 3, 14050 Berlin, Germany
[4]National Institute of Diabetes and Digestive and Kidney Diseases, National Institute of Health, Bethesda, Maryland 20892-1370

Objective: In the present study, we have addressed the question whether deletion of A_1 adenosine receptors affect glioblastoma – host interaction observed in A1AR deficient mice.

Methods: G261 glioblastoma cells were inoculated into $A_1AR^{-/-}$ mice and $A_1AR^{+/+}$ littermate controls. With this approach, we deleted the A_1AR in the host cells, but not in the inoculated GL261 glioblastoma cells. Animals were sacrificed 14 days after GL261 inoculation and the tumor area was determined double-blinded in axial section at the maximal diameter. Immunofluorescent triple labeling was carried out on 40-µm-free-floating sections using a spectral confocal microscope.

Results: The tumor size in $A_1AR^{-/-}$ mice was significantly larger as compared to $A_1AR^{+/+}$ mice (mean ± SE, 0.96 ± 0.09 mm for control (n=73); 1.69 ± 0.03 mm for $A_1AR^{-/-}$ mice (n=99)). To analyze the cell populations from the host in the vicinity of the tumor cells, we studied the distribution of microglial cells and astrocytes in $A_1AR^{-/-}$ and $A_1AR^{+/+}$ mice. Immunoreactivity for the macrophage / microglia marker Iba-1 revealed an accumulation of Iba-1 positive cells at the tumor border. In $A_1AR^{-/-}$ mice the density and number of Iba-1 positive cells was significantly higher as compared to wild-type littermates. No differences in the GFAP-positive cell population was observed comparing $A_1AR^{-/-}$ and $A_1AR^{+/+}$. In gelatin zymographies, we observed that
microglia abundantly release active MMP-2 after stimulation with glioma conditioned medium. The glioma-stimulated increase in MMP-2 activity was blunted by costimulation with 100 µmol/L adenosine.

Conclusion: These results imply that A_1AR modulate tumor growth and that microglial cells are the cellular candidates to mediate this effect.

List of Posters

1. CHARACTERIZATION OF A PUTATIVE TUMOR STEM CELL IN MALIGNANT BRAIN TUMORS
Acker, T.
Neuropathology, Edinger Institute, Deutschordenstr. 46, Frankfurt, eMail: till.acker@med.uni-frankfurt.de

2. DETECTION OF DE NOVO CHROMOSOMAL ABERRATIONS IN AN ESTHESIONEUROBLASTOMA - USING CYTOGENETIC AND MOLECULARCYTOGENETIC TECHNIQUES
Ahnert, P.; Koschny, R.; Krupp, W.; Meixensberger, J.; Holland, H.
IKIT/BBZ, Universität Leipzig, Johannisallee 30, Leipzig, eMail: peter.ahnert@gmx.net

3. ETS-1 DEPENDENT EXPRESSION OF TRANSFERRIN RECEPTORS IN GLIOMA MEDIATES IRON ACCUMULATION, REACTIVE OXYGEN SPECIES GENERATION AND TUMOUR PROGRESSION
Chirasani, S.; Glass, R.; Markovic, D.; Kettenmann, H.
Cellular Neurosciences, MDC, Rober Rossle Str-10, Berlin, eMail: sridhar.chirasani@mdc-berlin.de

4. DOWNREGULATION OF TGF-BETA AND ITS RECEPTORS BY THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA(PPAR-GAMMA) AGONIST TROGLITAZONE IN GLIOMA CELLS
Coras, R.; Blümcke, I.; Hahnen, E.
Department of Neuropathology, University of Erlangen, Krankenhausstraße 8-10, Erlangen, eMail: roland.coras@gmx.de

5. CB2 EXPRESSION IN ADULT AND PEDIATRIC PRIMARY BRAIN TUMORS AND EFFICACY OF SYNTHETIC CANNABINOIDS TO INDUCE APOPTOSIS IN TP53-NULL AND/OR PTEN-NULL HUMAN GLIOBLASTOMA CELLS
Ellert-Miklaszewska, A.; Grajkowska, W.A.; Gabrusiewicz, K.; Owczarek, D.; Danilkiewicz, M.; Konarska, L.; Kaminska, B.
Laboratory of Transcription Regulation, Nencki Institute of Experimental Biology, 3, Pasteur str., Warsaw, eMail: aellert@nencki.gov.pl

6. INVOLVEMENT OF HIF-1 IN DESFERRIOXAMINE (DFO) INDUCED INVASION OF GLIOBLASTOMA CELLS
Elstner, A.; Holtkamp, N.; von Deimling, A.
Neuropathology, Universitätsmedizin Charité Berlin, CVK, Augustenburger Platz 1, Berlin, eMail: anja.elstner@charite.de

7. INHIBITION OF GLIOMA INVASION AND TUMOR-INDUCED CELL DEATH BY TARGETED XCT INTERFERENCE
Eyupoglu, I.Y.; Heckel, A.; Hahnen, E.; Blumcke, I.; Ganslandt, O.; Buchfelder, M.; Nimsky, C.; Savaskan, N.E.
Department of Neurosurgery, University of Erlangen-Nuremberg, Schwabachanlage 6, Erlangen, eMail: eyupoglu@gmx.net

8. CANNABINOIDS INDUCE APOPTOSIS OF GLIOMA CELLS IN VITRO AND TUMOR REGRESSION IN VIVO
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Department of Cell Biology, The Nencki Institute of Experimental Biology, Pasteur 3 Street, Warsaw, eMail: k.gabrusiewicz@nencki.gov.pl

9. INTRACRANIAL EXTRASKELETAL MESENCHYMAL CHONDROSARCOMA OF A 13-YEAR-OLD BOY
Hahn,G.; Geiger,K.; von der Hagen,M.
Uniklinikum Carl Gustav Carus Dresden, Institut und Poliklinik für Radiologische Diagnostik, Fetschertstrasse 74, Dresden, eMail: Gabriele.Hahn@uniklinikum-dresden.de

10. EXPRESSION AND FUNCTIONAL ROLE OF CANNABINOID RECEPTORS IN HUMAN ASTROGLIAL TUMORS
Held-Feindt, J.; Dörner, L.; Sahan, G.; Mehdorn, H.M.; Mentlein, R.
Neurosurgery, UK S-H, Campus Kiel, Schittenhelmstr. 10, Kiel, eMail: held-feindtj@nch.uni-kiel.de

11. DETECTION OF DE NOVO CHROMOSOMAL ABERRATIONS IN AN ESTHESIONEUROBLASTOMA USING CYTOGENETIC AND MOLECULARCYTOGENETIC TECHNIQUES
Holland, H.; Koschny, R.; Krupp, W.; Meixensberger, J.; Ahnert, P.
Biotechnical-Biomedical Centre (BBZ) and Institute of Clinical Immunology and Tr, Johannisallee 30, Leipzig, eMail: Heidrun.Holland@medizin.uni-leipzig.de

12. CHARACTERIZATION OF THE AMPLICON ON CHROMOSOMAL SEGMENT 4Q12 IN GLIOBLASTOMA MULTIFORME

Holtkamp, N.; Ziegenhagen, N.; Zietsch, J.; Malzer, E.; Hartmann, C.; Giese, A.; von Deimling, A.
Neuropathology, Charité – Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin, eMail: nikola.holtkamp@charite.de

13. NECROTIC MENINGIOMA: CHARACTERISTIC IMAGING FINDINGS IN DIFFUSION MRI AND MR SPECTROSCOPY

Ivens, S.; Merkin, V.; Kaniano, E.B.; Shelef, I.
Institute of Physiology, Charité University Medicine, Tucholskystr. 2, Berlin, eMail: sivens@gmail.com

14. INTERPHASE IN SITU HYBRIDIZATION ANALYSES OF CHROMOSOME 1 IN A SERIES OF PROGRESSIVE MENINGIOMAS

Kim, Y.J.; Bochem, N.; Mehraein, Y.; Ketter, R.; Henn, W.; Zang, K.D.; Feiden, W.
Institute of Neuropathology, Saarland University, Kirrbergstr. Geb.90.3, Homburg, eMail: yoo.jin.kim@uniklinikum-saarland.de

15. GLIOMAS STIMULATE MT1-MMP OVER-EXPRESSION IN MICROGLIA

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Neurosurgery, Helios Clinics, Hobrechtsfelder Chaussee, Berlin, eMail: DMarkovic@berlin.helios-kliniken.de

16. ENDOGLIN (CD105) OVEREXPRESSION ENHANCES MIGRATORY AND INVASIVE PROPERTIES OF MDA-MB-231 BREAST CANCER CELLS AND MODULATES TGF-BETA SIGNAL TRANSDUCTION

Oxmann, D.; Held-Feindt, J.; Stark, A.; Mentlein, R.
Anatomisches Institut, Christian-Albrechts-Universität zu Kiel, Otto-Hahn-Platz 8, Kiel, eMail: d.oxmann@anat.uni-kiel.de

17. ADULT HUMAN MESENCHYMAL STEM CELLS ARE RECRUITED BY INTRACRANIAL GLIOMAS AND INTEGRATED INTO THE TUMOR VASCULATURE

Schichor, Ch.; Birnbaum, T.; Schnell, O.; Grau, S.; Trillsch, F.; Loos, S.; Krebs,B.; Tonn, J.-C.; Nelson, P.; Goldbrunner, R.
University of Munich, LMU, Neurosurgical Clinic, Marchioninistr.15, 81377 Munich, eMail: schichor@web.de

18. CHARACTERISATION OF A PUTATIVE TUMOR STEM CELL LINE DERIVED FROM THE ADULT RAT SUBVENTRICULAR ZONE

Siebzehnrübl, F.A.; Müller, D.; Pflanzner, T.; Buslei, R.; Eyüpoglu, I.Y.; Hahnen, E.; Blümcke, I.
Dept. of Neuropathology, University Erlangen, Krankenhausstr. 8 - 10, Erlangen, eMail: fs@neuropatho.imed.uni-erlangen.de

19. INHIBITION OF VINCRISTINE EFFECT BY BLOCKING GLUCOSE TRANSPORTER 1 IN HUMAN GLIOBLASTOMA CELL LINES

Stockhammer, F.; Elstner, A.; von Deimling, A.
Neurosurgery, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin, eMail: florian.stockhammer@charite.de

20. IRRADIATION AND HYPOXIA ENHANCE GLIOMA-MEDIATED ATTRACTION OF HEMATOPOIETIC PROGENITOR CELLS

Ghazaleh Tabatabai[1], Brigitte Frank[1], Robert Möhle[2], Michael Weller[1],a Wolfgang Wick[1,3]
[1]Laboratory of Molecular Neuro-oncology, Department of General Neurology and Hertie Insitute for Clinical Brain Resarch, [2]Department of Internal Medicine II (Hematology), University of Tübingen, Tübingen, Germany

21. APOPTOSIS-BASED TREATMENT OF GLIOBLASTOMA WITH ABT-737, A NOVEL SMALL MOLECULE INHIBITOR OF BCL-2 FAMILY PROTEINS

Tagscherer, K.; Böck, B.; Eckert, A.; Herold-Mende, C.; Wiestler, O.; Roth, W.
Molecular Neuro-Oncology, German Cancer Research Center, Im Neuenheimer Feld 280, Heidelberg, eMail: k.tagscherer@dkfz-heidelberg.de

22. FREQUENT EPIGENETIC SILENCING OF NPTXII IN GLIOBLASTOMAS.

Vaitkine, P.; Mueller, W.; Laß, U.; Ehrich, M.; Louis, D.N.; von Deimling, A.
Neuropathology, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, Berlin, eMail: wolf.mueller@charite.de

23. LD-TYPE CYCLINS CONTROL THE ANTI-TUMOURIGENIC RESPONSE OF NEURAL PRECURSOR CELLS AGAINST GLIOMAS

Wälzlein, J-H.; Glass, R. ; Kaczmarek, L.; Kaminska, B.; Nuber, U.; Kettenmann, H.
Cellular Neurosciences, Max-Delbrueck-Centrum, Robert-Roessle-Strasse 10, Berlin, eMail: j.waelzlein@mdc-berlin.de

24. FASL AS A CROSSTALK MOLECULE IN GLIOMA-MICROGLIA INTERACTIONS

Wisniewski, P.; Kaminska, B.
Department of Cell Biology, The Nencki Institute of Experimental Biology, Pasteur 3, Warsaw, eMail: p.wisniewski@nencki.gov.pl

Abstracts of Poster Presentations

1. CHARACTERIZATION OF A PUTATIVE TUMOR STEM CELL IN MALIGNANT BRAIN TUMORS

Acker, T[1]; Wirta, V[2]; Schaenzer, A[1], Plate, K.H.[1], Lundeberg, J[2], Frisén, J[3],
[1]Edinger Institute, Neuropathology, Frankfurt, Germany; [2]Dept. of Biotechnology; KTH, Stockholm, Sweden [3]Karolinska Institute, CMB, Stockholm, Sweden

Self-renewal and multi-potency are by definition essential characteristics of stem cells. On the other hand, dysregulated self-renewal is a feature of tumor growth. The aberrant activation or dysregulation of organotypic stem cell pathways has been classically associated with tumor growth and progression. Indeed several recent publications point to the existence of a tumor stem cell and suggest remarkable parallels between stem cell and tumor cell biology.The isolation of stem cells based on the capacity to efflux Hoechst 33342 is an efficient method to purify stem cells from different tissues, characterizing the side population (SP). We could identify a SP encompassing 0,1%-1,1% of all cells in different glioblastomas. The SP fulfilled tumor stem cell characteristics, i.e. SP cells were able to self-renew, gave rise to a more differentiated progeny and formed tumors in a xenograft model in vivo. In analogy to neural stem cells, glioblastoma cells when cultured in serum-free neurosphere medium grew as tumor spheres over several passages. Both, growth factors (e.g. EGF, FGF) as well as transcription factors (HIF-hypoxia inducible factor) modulated the size of the SP. Interestingly, SP and non-SP are characterized by a different set of genes as determined by transcriptional profiling via microarray analysis hinting towards distinct genetic programs operative in the two populations.In summary our results support the validity of the tumor stem cell concept and the existence of a differentiation hierarchy in tumors. Further understanding to what degree tumor stem cells and organ-specific stem cells make use of related signalling mechanisms to regulate self-renewal, proliferation and differentiation may provide deeper insights into tumor pathophysiology and may, in addition, help as a “Spin-off” to understand processes of physiological stem cell homeostasis.

2. DETECTION OF DE NOVO CHROMOSOMAL ABERRATIONS IN AN ESTHESIONEUROBLASTOMA - USING CYTOGENETIC AND MOLECULARCYTOGENETIC TECHNIQUES

Ahnert, P.; Koschny, R.; Krupp, W.; Meixensberger, J.; Holland, H.
Universität Leipzig, IKIT/BBZ, Johannisallee 30, 04103 Leipzig, peter.ahnert@gmx.net

Esthesioneuroblastoma is a malignant neuroectodermal tumor originating from olfactory epithelial cells in the nasal vault. Due to the rarity of this tumor entity cytogenetic data are very limited. Therefore, we performed comprehensive cytogenetic analyses of an esthesioneuroblastoma, Hyams grade III-IV, using trypsin-Giemsa staining (GTG-banding), multicolor fluorescence in situ hybridization (M-FISH), and locus-specific FISH complemented by molecular karyotyping using high density SNP-arrays. GTG-banding of 25 metaphases revealed 54 structural intrachromosomal aberrations, predominantly located on 2q, 6q, 21q, and 22q, which were confirmed by FISH analysis. Interestingly, we found two novel, so far not described deletions, del(2)(q37) and del(21)(q22). Using GTG-banding, locus-specific FISH, and M-FISH we detected frequent numerical changes of chromosomes 5, 17, 19, and 22, as well as trisomy 8 at low frequency. Applying SNP-array karyotyping we confirmed the chromosomal aberrations del(2)(q37.3), del(3)(q27.2), del(10)(q26.11), chromosomal imbalance on 17q, del(21)(q22), and revealed a number of so far unknown aberrations (gain of 2q14.3, 13q33.3, and 13q34). While the cytogenetically revealed low frequency mosaic del(6)(q22q24) was not visible using SNP-array karyotyping, some of the smaller imbalances (SNP-array data) could not have been detected by classical cytogenetic analysis. Therefore, our study supports the usefulness of applying complementary methods for cytogenetic analysis.

3. ETS-1 DEPENDENT EXPRESSION OF TRANSFERRIN RECEPTORS IN GLIOMA MEDIATES IRON ACCUMULATION, REACTIVE OXYGEN SPECIES GENERATION AND TUMOUR PROGRESSION.

Sridhar Chirasani, Rainer Glass, Darko Markovic, Helmut Kettenmann
Department of Cellular Neurosciences, Max-Delbrück Center for Molecular Medicine (MDC) Berlin-Buch, Germany.

Glioblastomas are the most common and devastating primary brain tumours. These tumours are long known to overexpress transferrin receptors (TfRs), which mediate the cellular uptake of iron. In pathologic brain tissue, iron can accumulate intracellularly and causes deleterious effects, presumably through the generation of reactive oxygen species (ROS) via the Fenton reaction. Tumour cells appear to generate high ROS levels to maintain their malignant potential. Here, we show that the transcription factor Ets-1 regulates the expression of TfRs and thereby mediates iron accumulation, ROS generation and tumour progression in human glioblastoma cell lines. Blocking Ets-1 activity by stable transfection of the dominant negative form of Ets-1 (Ets-1Dn) in the human glioblastoma cell line U373 attenuated the expression of transferrin receptors, changed cell morphology, decreased their proliferation, and significantly attenuated the migration of glioma cells. Concentration of free iron (Fe^{+2}), as measured by iron imaging using Phen Green Sk, and ROS levels measured by using H_2DCFDA and hydroethedine, were both down regulated in U373 Ets-1DN cells compared to wild type U373 cells. Stable overexpression of TfRs in Ets-1Dn cells compensated the effect of blocking Ets-1 activity. The cells had similar levels of free iron, ROS and proliferation as wild-type glioma cells. Moreover, overexpression of *TfR* by transfection into a human astrocytoma line (1321N1) with low malignancy strongly increased the proliferation of these cells. Ets-1 binding to the TfR promoter region is essential for driving the expression of TfR in human glioma cells as demonstrated by reporter gene activation assays. We conclude that the altered expression of transferrin receptors in human glioma cells, regulated by Ets-1, mediates ROS signalling, proliferation and migration and thereby enhances glioma progression.

4. DOWNREGULATION OF TGF-Â AND ITS RECEPTORS BY THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR γPPAR-γ) AGONIST TROGLITAZONE IN GLIOMA CELLS

Roland Coras1, Annett Hölsken1, Ilker Y Eyüpoglu2, Sebastian Seufert3, Jan Hauke3, Martin Reichel4, Christian Tränkle5, Florian A Siebzehnrübl1, Rolf Buslei1, Ingmar Blümcke1 and Eric Hahnen1, 3
1Department of Neuropathology, University of Erlangen, Germany; 2 Department of Neurosurgery, University of Erlangen, Germany; 3Institute of Human Genetics; Institute of Genetics, and Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany, 4Department of Experimental Medicine II, University of Erlangen, Germany; 5Department of Pharmacology & Toxicology, Institute of Pharmacy, University of Bonn, Germany;

The transforming growth factor beta (TGF-β)-signaling pathway is involved in rapid proliferation and brain invasion of malignant gliomas. Here, we identify the peroxisome proliferator-activated receptor γ (PPAR-γ agonist Troglitazone (TRO) as potent down-regulator of TGF-β and its receptors in glioma cell lines obtained from rat, mouse or human species. Treatment of rat F98 glioma cells with TRO significantly reduced cell proliferation (IC90 = 65 µM) and was mediated by G0/1 cell cycle arrest. Implantation of eGFP transfected F98 glioma cells into slice cultures of rat brain confirmed the cytostatic effect of TRO without neurotoxic damage to the organotypic neuronal environment in a dose escalation up to 130 µM. Interestingly, also diffuse migration of glioma cells was arrested in the organotypic environment following TRO application and was confirmed either in a wound healing or Boyden chamber migration assay. TRO may thus present a promising candidate drug targeting TGF-β mediated tumor progression in malignant gliomas.

5. CB2 EXPRESSION IN ADULT AND PEDIATRIC PRIMARY BRAIN TUMORS AND EFFICACY OF SYNTHETIC CANNABINOIDS TO INDUCE APOPTOSIS IN TP53-NULL AND/OR PTEN-NULL HUMAN GLIOBLASTOMA CELLS

Aleksandra Ellert-Miklaszewska [1,3], Wieslawa A. Grajkowska [2], Konrad Gabrusiewicz [3], Dorota Owczarek[3], Malgorzata Danilkiewicz [3], Liliana Konarska [1], Bozena Kaminska [3]

[1]Dept. of Biochemistry and Clinical Chemistry, Medical University, Warsaw, Poland [2]Dept. of Pathology, Children's Memorial Health Institute, Warsaw, Poland

[3]Lab. of Transcription Regulation, Nencki Institute of Experimental Biology, Warsaw, Poland

Cannabinoids, originally derived from *Cannabis sativa*, as well as endogenous and synthetic agonists of CB1 and CB2 cannabinoid receptors, have been recently extensively studied as potential antitumoral agents towards various cancers, including gliomas. Since the known psychotropic effects of cannabinoids are mediated largely by CB1 receptors, we sought to determine the CB2 expression in paraffin sections from various adult and pediatric primary brain tumors as well as the efficacy of selective CB2 receptor activation to kill human glioblastoma cells. Moreover, we examined whether synthetic cannabinoids with different receptor specificity are able to induce apoptosis in drug-resistant *TP53*-null and/or *PTEN*-null glioma cells. As investigated by immunohistochemistry, most of the analyzed human brain tumors, primarily glioblastomas, expressed significant levels of the CB2 receptor. The extent of CB2 expression depended on histopathological origin of the tumor and correlated with tumor malignancy. In the cell culture experiments, both cannabinoids, WIN55,212-2 (non-selective CB1/CB2 agonist) and JWH133 (CB2-selective agonist), induced activation of caspase cascade and DNA fragmentation in established cell lines - T98G, U373MG, U87MG, LN229, and primary glioma cultures. Apoptosis was observed even in the cells with mutated tumor suppressors *TP53* or/and *PTEN*, with the only exception that sensitivity to JWH133 treatment was obviously related to CB2 expression. Altogether, CB2 receptor expression in adult and pediatric glioblastomas, and susceptibility of human glioblastoma cells to synthetic cannabinoids despite mutations contributing to general apoptosis resistance, allow one to view cannabinoid-based therapies as promising anti-glioma strategy.

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6. INVOLVEMENT OF HIF-1 IN DESFERRIOXAMINE (DFO) INDUCED INVASION OF GLIOBLASTOMA CELLS

Anja Elstner, Nikola Holtkamp, Andreas von Deimling

Institute of Neuropathology, Charité-Universitätsmedizin Berlin, CVK, 13353 Berlin, Germany

Glioblastoma multiforme are highly invasive brain tumors. Experimental approaches focus on unravelling the mechanisms of invasion, this being a major reason for the poor prognosis of these tumors. Our previous results hinted towards involvement of the iron metabolism in invasion. In this study we examined the effect of iron depletion on the invasive phenotype of glioblastoma cells. Transwell Matrigel invasion assays were used to monitor iron-dependent invasion of human glioblastoma cell lines U373MG and DBTRG05MG. Intracellular iron concentrations were modulated by applying desferrioxamine (DFO) and ferric ammonium citrate (FAC). We detected enhanced invasion of glioblastoma cells upon DFO induced iron depletion. Treatment of cells with FAC strongly inhibited invasion. DFO treatment resulted in hypoxia-inducible factor 1 (Hif-1) mediated induction of urokinase plasminogen activator receptor and matrix metalloproteinase 2. Further, RNA interference-mediated repression of urokinase plasminogen activator receptor inhibited DFO induced invasion. Our data demonstrate a direct effect of DFO on Hif-1 expression resulting in activation of factors associated with ECM degradation and invasion of glioma cells. These findings caution on utilization of DFO and other iron chelators in the treatment of tumors with invasive potential.

7. INHIBITION OF GLIOMA INVASION AND TUMOR-INDUCED CELL DEATH BY TARGETED XCT INTERFERENCE

Eyupoglu, I.Y. [1]; Heckel, A. [1]; Hahnen, E. [2]; Blumcke, I[3].; Ganslandt, O. [1]; Buchfelder, M. [1]; Nimsky, C. [1]; Savaskan, N.E. [4]

[1]Department of Neurosurgery, University of Erlangen-Nuremberg, Germany; [2]Institute of Human Genetics, Institute of Genetics and Center for Molecular Genetics Cologne (CMMC), University of Cologne, Germany; [3]Department of Neuropathology, University of Erlangen-Nuremberg, Germany; [4]Division of Cellular Biochemistry, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

A hallmark of invasive brain tumors such as glioblastoma multiforme is the massive cell death that occurs in adjacent brain parenchyma. The mechanism by which malignant gliomas cause cell death along the growing tumor margins is unclear, however, it is thought that extracellular neurotoxic factors play an important role in this process. To address this issue, we developed a technique for tracking glioma cell expansion in an organotypic brain environment using real-time fluorescence microscopy. We identified that the excitatory neurotransmitter glutamate is released by glioma cells at neurotoxic concentrations during brain invasion. In line with these results, abrogation of glutamate secretion from glioma cells blocked neuronal cell death. Analysis of glutamate transporters revealed further that the cystine-glutamate exchanger xCT (SLC7A11) is mainly expressed in gliomas. Moreover, siRNA-mediated knock down of the cystine/glutamate transporter xCT in glioma cells implantated into brain tissue prevented both peritumoral cell death and glioma expansion. Thus, these results show that xCT is indispensable for malignant glutamate secretion and glioma infiltration. These data support the concept that peritumoral cell death is dependent upon xCT-mediated glutamate secretion. Therefore, xCT presents a potential prime target for therapeutic intervention of brain malignancies.

8. CANNABINOIDS INDUCE APOPTOSIS OF GLIOMA CELLS *IN VITRO* AND TUMOR REGRESSION *IN VIVO.*

Gabrusiewicz Konrad[1], Ellert-Miklaszewska Aleksandra[1,3], Grajkowska Wieslawa[4], Figiel-Ozog Izabela[2], Konarska Liliana[3], Kaminska Bozena[1]

[1]Laboratory of Transcription Regulation, Department of Cell Biology, Nencki Institute, Warsaw, Poland;

[2]Laboratory of Mechanisms of Neuroprotection and Neurodegeneration, Department of Molecular and Cellular Neurobiology, Nencki Institute, Warsaw

[3]Department of Biochemistry and Clinical Chemistry, Medical University of Warsaw;

[4]Department of Neurosurgery, Children's Memorial Health Institute, Warsaw;

Natural cannabinoids, as well as their endogenous and synthetic counterparts, generate a wide range of central and peripheral effects mediated mostly through cannabinoid receptors CB1 and CB2. Antiproliferative effects of cannabinoids have been reported in various cancer cells, including gliomas, a class of highly malignant brain tumors. THC and synthetic cannabinoid receptor agonists have been shown to inhibit the growth of glioma cells *in vitro* and to induce a considerable regression of malignant gliomas in rats after intratumoral drug injection. The aim of our study was investigate the effectiveness of an intraperitoneally administered synthetic cannabinoid in reducing the tumor growth in vivo and to evaluate the mechanism of glioma cell death in culture. Synthetic cannabinoids, both nonselective and CB2-selective decreased viability of C6 glioma cells, induced caspase activation and poly(ADP-ribose)polymerase (PARP) cleavage. JWH-133 proved to be less toxic than WIN-55,212-2 in mixed neuronal-glial culture, which prompted us to use CB2-selective agonist in *in vivo* glioma model. Intraperitoneal administration of the cannabinoid resulted in decrease of tumor volume in drug-treated rats when compared with the control animals. JWH133 is able to induce apoptosis in glioma cells in vitro, while having no cytotoxic influence on non-transformed cells. Antitumoral effectiveness of a systemically injected cannabinoid may be of clinical relevance.

9. INTRACRANIAL EXTRASKELETAL MESENCHYMAL HONDROSARCOMA OF A 13-YEAR-OLD BOY

Hahn,G.; Geiger,K.; von der Hagen,M.
Uniklinikum Carl Gustav Carus Dresden

We describe a patient with an intracranial extra skeletal mesenchymal chondrosarcoma an unusual neoplasm of the deep soft tissues of the extremities. Central nervous system mesenchymal chondrosarcomas are rare malignant tumors that constitute a separate entity from the classical chondrosarcoma and myxoid variant. Clinical behaviour of central nervous system chondrosarcomas is still unknown. The case of a 13-year-old boy with mesenchymal chondrosarcoma which seemed to arise from the brain is presented here. The tumor involved the left lateral ventricle and the left cerebral hemisphere and reached the left calvarium without bone destruction.

10. EXPRESSION AND FUNCTIONAL ROLE OF CANNABINOID RECEPTORS IN HUMAN ASTROGLIAL TUMORS

Janka Held-Feindt , J.*; Dörner, L.*; Sahan, G.*; Mehdorn, H.M.*; Mentlein, R.**
* *epartment of Neurosurgery, Universitätsklinikum Schleswig-Holstein Campus Kiel, Kiel, Germany, **Department of Anatomy, University of Kiel, Kiel, Germany*

In animal models, cannabinoids are reported to inhibit the growth of tumors, including gliomas. These effects have been claimed to be mediated via cannabinoid receptors 1 and 2 (CB1, CB2). To elucidate a possible relevance for treatment of human gliomas, we investigated receptor subtype expression in surgical material of solid human astrocytomas, gliomas and in cultivated glioma cells by real-time RT-PCR, Western blot, immunohistochemistry and assayed their functionality. In normal brain, cultivated glioma cells and solid tumors, CB1 mRNA was higher expressed than CB2. Expression of both receptor subtypes was unrelated to malignancy, varied between patients, and was not significantly increased in relation to normal brain tissues. In normal brain, CB1 protein was localized on astroglial and other cell types; in gliomas, it was found on astroglial / glioma cells. CB2 protein was detected on microglial cells / macrophages, rarely on astroglial cells.

11. DETECTION OF DE NOVO CHROMOSOMAL ABERRATIONS IN AN ESTHESIONEUROBLASTOMA USING CYTOGENETIC AND MOLECULARCYTOGENETIC TECHNIQUES

Holland, H.; Koschny, R.; Krupp, W.; Meixensberger, J.; Ahnert, P.
Biotechnical-Biomedical Centre (BBZ) and Institute of Clinical Immunology and Transfusion Medicine, Faculty of Medicine, University of Leipzig, Leipzig, Germany

Esthesioneuroblastoma is a malignant neuroectodermal tumor originating from olfactory epithelial cells in the nasal vault. Due to the rarity of this tumor entity cytogenetic data are very limited. Therefore, we performed comprehensive cytogenetic analyses of an esthesioneuroblastoma, Hyams grade III-IV, using trypsin-Giemsa staining (GTG-banding), multicolor fluorescence in situ hybridization (M-FISH), and locus-specific FISH complemented by molecular karyotyping using high density SNP-arrays. GTG-banding of 25 metaphases revealed 54 structural intrachromosomal aberrations, predominantly located on 2q, 6q, 21q, and 22q, which were confirmed by FISH analysis. Interestingly, we found two novel, so far not described deletions, del(2)(q37) and del(21)(q22). Using GTG-banding, locus-specific FISH, and M-FISH we detected frequent numerical changes of chromosomes 5, 17, 19, and 22, as well as trisomy 8 at low frequency. Applying SNP-array karyotyping we confirmed the chromosomal aberrations del(2)(q37.3), del(3)(q27.2), del(10)(q26.11), chromosomal imbalance on 17q, del(21)(q22), and revealed a number of so far unknown aberrations (gain of 2q14.3, 13q33.3, and 13q34). While the cytogenetically revealed low frequency mosaic del(6)(q22q24) was not visible using SNP-array karyotyping, some of the smaller imbalances (SNP-array data) could not have been detected by classical cytogenetic analysis. Therefore, our study supports the usefulness of applying complementary methods for cytogenetic analysis.

12. **CHARACTERIZATION OF THE AMPLICON ON CHROMOSOMAL SEGMENT 4Q12 IN GLIOBLASTOMA MULTIFORME**

Nikola Holtkamp1, Nicolas Ziegenhagen1, Jan Zietsch1, Elke Malzer1, Christian Hartmann1, Alf Giese2 and Andreas von Deimling1.
1. Institute of Neuropathology, Charité – Universitätsmedizin Berlin, Germany 2. Department of Neurosurgery, Georg-August-Universität Göttingen, Germany.

A subset of glioblastomas (GBM) carries gene amplifications on chromosomal segment 4q12. To characterize this amplicon in detail we analyzed 87 gliomas and 13 glioma cell cultures. We applied multiplex ligation-dependent probe amplification (MLPA) to determine the gene dosage of *PDGFRA, KIT* and *KDR* and the flanking genes *UPS46, RASL11B, LNX1, CHIC2, SEC3L1* and *IGFBP7*. The amplicon was highly variable in size and copy number and extended over a region of up to 5 Mb. Amplifications on 4q12 were observed in 15% of GBM (n=65) and 23% of GBM cell cultures (n=13) but not in 22 other gliomas. We analyzed transcription and translation of some genes within this amplicon. Gene amplification generally correlated with high transcript levels but did not necessarily result in increased protein levels. However, we detected frequent expression of proteins encoded by *PDGFRA*, *KIT* and *KDR* in GBM and GBM cell cultures independent of the amplification status. Sunitinib, a new drug that targets the kinases encoded by *PDGFRA, KIT* and *KDR*, is currently tested on our characterized GBM cell lines. First results demonstrate high sensitivity to sunitinib indicating that future treatment of GBM patients may include drugs targeting multiple kinases encoded by genes on chromosomal segment 4q12. We will now investigate, whether molecular determinants like gene dosage, protein expression levels or activation of signal transduction pathways will predict inhibitory concentration 50 (IC50) of sunitinib.

13. NECROTIC MENINGIOMA: CHARACTERISTIC IMAGING FINDINGS IN DIFFUSION MRI, AND MR SPECTROSCOPY

Sebastian Ivens1*,2, Vladimir Merkin3, Emanuela Bastians Kaniano4, and Ilan Shelef1*
1) Department of Radiology and Neuro[rad]*iolgy Unit, Soroka*[a] *University Medical Center, Ben-Gurion University, Beer-Sheva, Israel 2) Institut für Physiologie, Charité University Medicine, Berlin, Germany 3) Department of Neurosurgery Soroka University Medical Center, Ben-Gurion University, Beer-Sheva, Israel 4) Department of Pathology Soroka University Medical Center, Ben-Gurion University, Beer-Sheva, Israel,*

Meningiomas are the most common extraaxial tumors, accounting for approx. 15% of intracranial neoplasm. They are usually benign tumors with a typical appearance in MRI. However, a small fraction of these tumors may develop malignant dysplasia or present untypical imaging features and can be easily confused with metastases, malignant astrocytomas, inflammation or infarction in routine MRI protocols. Use of diffusion weighted imaging (DWI) and apparent diffusion coefficient (ADC) maps, as well as MR spectroscopy (MRS) can provide useful additional information to differentiate between these pathologies. Here, we present a case of histologically confirmed necrotic meningioma. We demonstrate the characteristic findings of this uncommon tumor in conventional MR, as well as in DWI and and MRS.

14. INTERPHASE IN SITU HYBRIDIZATION ANALYSES OF CHROMOSOME 1 IN A SERIES OF PROGRESSIVE MENINGIOMAS

Kim Yoo-Jin[1], Bochem Nora[1], Mehraein Yasmin[2], Ketter Ralf[3], Henn Wolfram[2], Zang Klaus D.[3], Feiden Wolfgang[1]
[1]Institute of Neuropathology, [2]Institute of Human Genetics, [3]Department of Neurosurgery, Medical School, Saarland University

The hypothesis of clonal evolution in meningiomas suggests the stepwise progression of genetic changes from low-grade toward a higher-grade tumor. This postulate is based on empiric data derived from analyses of cytogenetic findings from different types of tumors of different grades and in different individuals.

Monosomy 22 is the characteristic and most frequent cytogenetic finding in meningiomas. The progression from common-type to atypical and anaplastic meningioma is characterized by two different cytogenetic events: Firstly, an increasing hypodiploidy with mostly a typical pattern of clonal evolution and secondly, partial or complete loss of the short arm of one chromosome 1 (1p-). In this study, the genetic evolution focused on chromosome 1 during tumor progression was investigated in a group of seven patients in whom the meningiomas showed histopathologically confirmed progression toward a higher grade of malignancy. Fluorescence in situ hybridization was performed in the primary lower-grade tumors as well as their successive higher-grade recurrent tumors. Without exception, all higher-grade recurrent tumors (atypical or anaplastic meningiomas) showed deletions on the short arm of one chromosome 1. Interestingly, 1p- was also present already ab initio in all seven primary tumors despite their lower histopathological grade. These findings confirm the previous reported assumption, that 1p- represents an initial and decisive genetic event in the tumor progression of meningiomas and that 1p- indicates higher risk of recurrence and progression, even in histopathologically common-type meningiomas. Support: Wilhem-Sander Stiftung; Grant-No. 2005.164.1

15. GLIOMAS STIMULATE MT1-MMP OVER-EXPRESSION IN MICROGLIA

Darko S. Markovic, Rainer Glass, Michael Synowitz M, Sridhar Chirasani and Helmut Kettenmann.

Rainer Glass, PhD, Max Delbrück Center, Cellular Neuroscience, Robert Rössle Str 10, DE-13125 Berlin, Germany, Tel: +49 30 9406 3260, Fax: +49 30 9406 3819

Gliomas represent the most frequent type of human brain tumor and their strong invasiveness is a significant clinical problem. Microglia, the immunocompetent cells of the brain, contribute significantly to the tumor mass and are potential interaction partners of the glioma cells. Brain slice experiments showed that the presence of microglia within a gliom has a tumour promoting effect. Cell culture experiments revealed, that soluble factors released from glioma cells strongly stimulate the expression and activity of membrane type matrix metalloproteinase 1 (MT1-MMP), which in turn activates glioma-released pro-metalloprotease-2 (pro-MMP2). The induction of MT1-MMP in microglia is p38 MAPK dependant. Increased activity of microglial MT1-MMP accelerates tumor invasion, since MT1-MMP deficient animals inoculated with glioma developed significantly smaller tumors than heterozygous littermates. Our data indicate that glioma cells stimulate microglial cells to increase breakdown of extracellular matrix and thereby promote tumor-invasiveness.

16. ENDOGLIN (CD105) OVEREXPRESSION ENHANCES MIGRATORY AND INVASIVE PROPERTIES OF MDA-MB-231 BREAST CANCER CELLS AND MODULATES TGF-Â SIGNAL TRANSDUCTION

Oxmann, D; Held-Feindt, J; Stark, A; Mentlein, R.

Anatomisches Institut, Universität Kiel, Germany.

Introduction: A brain-seeking clone of MDA-MB-231 breast cancer cells exhibits elevated levels of Endoglin. To determine the relevance of altered Endoglin expression with regard to different aspects of cellular function and TGF-â signaling, we generated different endoglin clones of the parental cell line. Endoglin is an accessory component of the TGF-â receptor system and essential for vascular development.

Methods: MDA-MB-231 cells were stably transfected with endoglin expression and shRNA vectors. Invasiveness and cell migration was measured in Boyden chambers and in wound-induced migration assay. To monitor more complex cell functions we utilized a spheroid confrontation assay.

Results: Endoglin overexpression results in enhanced migration in Boyden chamber, whereas its downregulation reduces migration. The same consequence on migration was shown in assays for wound-induced migration and spheroid confrontation. After 6 hours almost all breast cancer cells had direct contact to U343 glioma cells, suggesting aimed migration of MDA-MB-231 cells towards U343 cells. When attached to U343 cells, endoglin overexpressing clones often show different morphology and growth in network-like patterns. In EMSA as well as in immunoblotting experiments we found an active Smad1 pathway only in mid-concentration TGF-â responses in endoglin overexpressing clones.

Conclusion: Endoglin is not only a component of the endothelial cell system, but also of tumor cells. In tumor cells Endoglin modulates smad-dependent TGF-â signaling, thereby regulating genes that are essential for vessel forming, for instance PAI-1. Stable transfection of endoglin in MDA-MB-231 cells complements the already existing set of endothelial marker proteins in this cell line and leads to changes of cell morphology resulting in vascular-like structures (vascular mimicry).

17. ADULT HUMAN MESENCHYMAL STEM CELLS ARE RECRUITED BY INTRACRANIAL GLIOMAS AND INTEGRATED INTO THE TUMOR VASCULATURE

Schichor Ch, , Birnbaum T., Schnell O., Grau S., Trillsch F., Loos S., B. Krebs., Tonn J-C., Nelson P., Goldbrunner R.

University of Munich, LMU, Neurosurgical Clinic, Marchioninistr.15,81377 Munich

OBJECTIVE: Much effort has been put into establishing human multipotent cells as carriers for malignant glioma therapy. On the other hand, the role of stem cells in initiation and growth of the tumor has been elucidated. We already described VEGF - dependent interaction of adult human mesenchymal stem cells (hMSC), which are easily available through bone marrow biopsy and glioma cells in vitro. Aim of our study was to characterize glioma-modulated invasive MSC- behaviour in vivo and distribution patterns in the glioma infiltrated brain.

METHODS: Human MSC were isolated from bone marrow biopsies carried out for haematological indications. Only early passages were used for the experiments. In an experimentally induced glioma (U373-GFP) infiltrated brain (T-cell deficient rats), hMSC (DiI) were implanted simultaneously. As control cells served Fibroblasts and immobilized hMSC. To exclude artificial attraction, a control incision was made. In a second setting a human MSC cell line, transfected with a RFP/Tie-2-promotor gene were given intraveneously. mMSC, which accumulated in the glioma (C6)-infiltrated brain were detected immunohistochemically. Tie2 induced expression of RFP allowed detection of those hMSC, which integrated into the endothelial lining of the vasculature.

RESULTS: Confocal microscopy revealed a colocalization of the hMSC and the infiltrating tumor. Fibroblasts as well as immobilized hMSC didn‘t show any localization in close vicinity of to the tumor, thereby excluding a passive transportation phenomenon of the hMSC within the glioma-infiltrated brain.Control incisions didn‘t show any hMSC-infiltration. Intraveneously administered hMSC showed extensive tropism to the glioma. The infiltrating borders of the tumor showed accumulation of hMSC as well as enhanced RFP expression of the mMSC, thereby indicating an integration into the tumor‘s neovascularization. This phenomenon was confirmed immunohistochemically.

CONCLUSIONS: hMSC show intensive tropism to invading glioma in vivo. Intraveneously circulating mesenchymal stem cells enrich within the tumor and seem to integrate into its vasculature. hMSC proved to be hopeful candidates for a future role as glioma treatment vectors.

18. CHARACTERISATION OF A PUTATIVE TUMOR STEM CELL LINE DERIVED FROM THE ADULT RAT SUBVENTRICULAR ZONE

FA Siebzehnrübl, D Müller, T Pflanzner, R Buslei, IY Eyüpoglu, E Hahnen and I Blümcke

Dept Neuropathology and Neurosurgery, Univ Erlangen-Nuremberg, Dept Genetics, Human Genetics and Center for Molecular Medicine Cologne, Univ Cologne

We present a novel cell line with putative tumor stem cell characteristics. Neural stem and progenitor cells were obtained from the subventricular zone of postnatal day 6 Wistar rats.

After eight cell culture passages a novel cellular phenotype emerged. Two more passages allowed to establish a homogenous cell line (termed R2303) that can be expanded in neurosphere conditions (N2 medium supplemented with EGF and FGF2) or as adherent cells (N2 medium supplemented with 10% FCS and retinoic acid). The population doubling time was about three days and the cells could be further passaged at least 100 times. Under adherent conditions, R2303 cells expressed markers of both glial (GFAP, vimentin, S100beta) and neuronal (betaIII tubulin, Map2) lineages as well as stem cells (Sox2, Nestin and Musashi1). A side population of approximately 0.2 % could be identified in R2303 cells, which was blocked by verapamil. Transplantation of R2303 cells into nude mice resulted in tumor formation, i.e. glioblastoma multiforme. In conclusion, this cell line may hint towards tumorigenesis from adult neural stem cells.

19. INHIBITION OF VINCRISTINE EFFECT BY BLOCKING GLUCOSE TRANSPORTER 1 IN HUMAN GLIOBLASTOMA CELL LINES

Florian Stockhammer[1], Anja Elstner[2], Andreas von Deimling [2]
[1]Klinik für Neurochirurgie, [2]Institut für Neuropathologie, Charité-Universitätsmedizin Berlin

Objective: Vincristine (VCR) is a widely used cytostatic agent in malignant gliomas. Due to its hydrophilic properties cellular uptake depends on transport. In gliomas this transport is unknown up to now. However, in other cancers glucose transporter 1 (GLUT1) was identified to contrive VCR efflux. As GLUT1 is overexpressed in hypoxic glioma regions, the VCR effect was investigated by selective GLUT1 blockage in cultured glioma cell lines.

Methods: U373MG, DBTRG05MG and a primary glioblastoma cell culture (BER) were incubated under hypoxic conditions (1% O_2). GLUT1 was depicted by Western blot analysis. Cell lines were treated either with 1 µg/ml VCR for 24 hours or in combination with 50 µM phloretin, a GLUT1 blocking agent. Further, we investigated the effect of phloretin withdrawal during VCR treatment. The cytotoxic effect was measured by cell counting using a CASY cell counter.

Results: Western blot analysis revealed a strong GLUT1 expression in all cell lines under hypoxic conditions. VCR led to a significant cell reduction ($p<0.001$). This cytotoxic effect could be inhibited by addition of phloretin ($p<0.001$), with almost normal cell growth compared to untreated control samples ($p>0.05$). Later phloretin removal by washing led to reduced cell growth again ($p<0.01$). In all findings cell size reversely correlated to cell count.

Conclusion: Blockade of GLUT1 reversibly inhibits vincristine cytotoxicity in cultured glioma cells. GLUT1 could be the major transport gateway for vincristine in gliomas. Further investigations should target GLUT1 to improve the effect of chemotherapy.

20. IRRADIATION AND HYPOXIA ENHANCE GLIOMA-MEDIATED ATTRACTION OF HEMATOPOIETIC PROGENITOR CELLS

Ghazaleh Tabatabai[1], Brigitte Frank[1], Robert Möhle[2], Michael Weller[1],a Wolfgang Wick[1,3]
[1]Laboratory of Molecular Neuro-oncology, Department of General Neurology and Hertie Insitute for Clinical Brain Resarch, [2]Department of Internal Medicine II (Hematology), University of Tübingen, Tübingen, Germany

In our previous work, we defined a pathway of transforming growth factor beta (TGF-b)- and stromal cell-derived factor-1/CXC chemokine ligand 12 (SDF-1a/CXCL12)-dependent migration of adult hematopoietic stem and progenitor cells (HPC) towards glioma cells *in vitro* and their homing to experimental gliomas *in vivo*. Irradiation is an essential standard therapy of glioblastoma patients and hypoxia is a critical aspect of the microenvironment of these tumors. Thus, we investigated the impact of irradiation and hypoxia on the attraction of HPC by glioma cells. Supernatants of irradiated or hypoxic LNT-229 glioma cells enhanced HPC migration *in vitro*. Reporter assays showed that the CXCL12 promoter activity is enhanced in LNT-229 cells at 24 h after irradiation at 8 Gy or after exposure to 1% oxygen for 12 h. The irradiation- and hypoxia-induced secretion of CXCL12 depended on hypoxia inducible factor-1 alpha (HIF-1a), but not on p53. The transcriptional induction of HIF-1a by hypoxia or irradiation requires an TGF-b signaling cascade. These findings delinate a novel stress signaling cascade in glioma cells involving TGF-b, HIF-1a and CXCL12. Cerebral irradiation of nude mice at 21 days after intracerebral implantation of LNT-229 glioma induces tumor satellite formation and enhances the glioma tropism of HPC to the tumor bulk and even to these satellites *in vivo*. We conclude from these data that the use of HPC as cellular vectors in the treatment of glioblastoma might be compatible with irradiation or other anti-angiogenic therapies inducing tumor hypoxia.

21. APOPTOSIS-BASED TREATMENT OF GLIOBLASTOMA WITH ABT-737, A NOVEL SMALL MOLECULE INHIBITOR OF BCL-2 FAMILY PROTEINS

Katrin Tagscherer, Barbara Böck, Anika Eckert, Christel Herold-Mende, Otmar Wiestler, Wilfried Roth
Molecular Neuro-Oncology, German Cancer Research Center, Im Neuenheimer Feld 280, 69120 Heidelberg

Defects in the apoptotic signaling cascades are one reason for the poor therapeutic response in malignant gliomas. As glioblastoma are characterized by high expression levels of anti-apoptotic Bcl-2 family proteins, we studied the effects of the novel Bcl-2 inhibitor, ABT-737, on malignant glioma cells. ABT-737 was cytotoxic in glioma cells at high nanomolar and low micromolar concentrations. ABT-737-induced cell death was characterized by the classical signs of apoptosis, such as activation of Caspase 3 and cleavage of the Caspase 3 substrate PARP. Co-treatment with ABT-737 and the death ligand TRAIL resulted in synergistic cytotoxicity in several cell lines. Moreover, ABT-737 sensitized glioma cells to the chemotherapeutic drugs vincristine and etoposide. To investigate whether anti-apoptotic proteins can inhibit ABT-737-induced cell death, glioma cell lines were stably transfected with Bcl-2 or Survivin. Over-expression of the anti-apoptotic protein Survivin did not inhibit ABT-737-induced cell death. In contrast, Bcl-2 over-expressing glioma cells exhibited enhanced sensitivity to ABT-737 accompanied by increased cleavage of Caspase 3. One reason for the Bcl-2-dependent sensitization to ABT-737 could be the displacement and release of the pro-apoptotic BAD protein from Bcl-2, caused by binding of ABT-737 to Bcl-2. In fact, co-immunoprecipitation experiments demonstrated that the binding of BAD to Bcl-2 is prevented in the presence of ABT-737. Taken together, ABT-737 preferably kills glioblastoma cells with high expression levels of the anti-apoptotic Bcl-2 protein. ABT-737 synergizes with the cytotoxic effects of anti-cancer agents and the death ligand TRAIL. Our findings suggest that ABT-737 is a promising novel drug for the experimental treatment of malignant glioma.

22. FREQUENT EPIGENETIC SILENCING OF *NPTXII* IN GLIOBLASTOMAS.

Vaitkine, P.[1]; Mueller, W.[1]; Laß, U.[1]; Ehrich, M.; Louis, D.N.[2]; von Deimling, A.[1]
[1] Institute of Neuropathology, Charite-Universitaetsmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany
[2] Department of Pathology, Cancer Center and Neurosurgical Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA.

It is agreed that epigenetic events in tumorigenesis constitute an alternative means of gene inactivation and may have an impact on tumor biology and outcome after treatment. In glioblastomas, *MGMT*- promoter methylation status may predict a more favorable outcome when treated with temozolomide. This finding stresses the importance to describe and unveil genes regulated by promoter hypermethylation in glioblastomas. We have recently identified a number of novel candidate genes, potentially regulated by promoter hypermethylation in glioblastomas, coupling pharmacological manipulation of methylation in short- term cultured primary gliomas with gene profiling. Here we present evidence for frequent epigenetic silencing of one of these novel candidates - *NPTX II* - in primary glioblastomas and the glioma

cell lines U87 and G98T. With RT-PCR, bisulfite- sequencing, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) and methylation specific PCR (MSP) we used a variety of independent techniques for validation. A better understanding of the glioblastoma epigenome not only will augment our knowledge of gliomagensis but may also pave the way for more tumor- tailored treatment regiments in the near future.

23. LD-TYPE CYCLINS CONTROL THE ANTI-TUMOURIGENIC RESPONSE OF NEURAL PRECURSOR CELLS AGAINST GLIOMAS.

Wälzlein, J-H.[1]; Glass, R[1]. ; Kaczmarek, L.[2]; Kaminska, B.[2]; Nuber, U[3]. and Kettenmann, H[1].
[1]Cellular Neuroscience Group, Max-Delbrueck Center for Molecular Medicine (MDC), Robert-Rössle-Str. 10, 13192 Berlin, Germany, [2]Department of Molecular and Cellular Neurobiology, Nencki Institute, 02-093 Warsaw, Poland, [3]Medical Faculty, University of Lund, Sweden

Adult neurogenesis takes place in two germinative centres of the brain, the subventricular zone (SVZ) and the dentate gyrus. In a previous study we demonstrated that neural precursor cells (NPCs) are attracted towards glioblastomas and have strong anti-tumourigenic effects by inducing glioblastoma cell death (Glass et al., 2005, J. Neurosci. 25:2637-2646). The increase in malignancy with age correlates with the age-dependent decline of NPCs in the brain. To study the influence of glioblastoma cells on NPC properties, we compared microarrays from NPCs alone or in coculture with glioblastoma cells. We observed an upregulation of several mitosis-related genes, especially of cyclin D1. RT-PCR of microdissected SVZ and immunohistochemistry of identified NPCs revealed that cyclin D1 and D2 are present in the SVZ of young (P30) mice, but only cyclin D2 remains expressed in fully adult (P90) animals. Cyclin D3 was virtually absent from the SVZ of P30 and P90 animals. P30 mice maintained the expression of cyclin D1 and D2 during tumour growth in the hemisphere ipsi- and contralateral to the tumour. Mice of P90 showed some reexpression of cyclin D1 in the ipsilateral hemisphere concomitant with a strong upregulation of p21 (waf1), a tumour suppressor protein regulated by p53, specifically in the tumour-exposed SVZ. As cyclin D2 is the predominant D-type cyclin in fully adult animals and is therefore exclusively responsible for adult neurogenesis, cyclin D2 knock-out animals have few proliferating NPCs. Glioma-injection into cyclin D2 (-/-) mice resulted in increased tumour size in both P30 and P90 mice as compared to wildtype animals. We conclude, that cell proliferation is necessary to recruit sufficient NPCs towards gliomas to mediate an anti-tumourigenic effect. NPCs in older animals have decreased proliferative potential due to a lack of cyclin D1 and even the attempt to re-express cyclin D1 is counteracted by a simultaneous rise in p21 (waf1).

24. FASL AS A CROSSTALK MOLECULE IN GLIOMA-MICROGLIA INTERACTIONS

Pawel Wisniewski, Bozena Kaminska
The Nencki Institute of Experimental Biology, Warsaw, Poland.

Background: Microglial cells have been shown recently to support tumor growth by promoting migration and proliferation of glioma cells. Microglia are the main source of cytotoxic cytokine – FasL in the CNS. Although FasL is able to induce apoptosis in Fas bearing cells, it may also play different, non-apoptotic roles in many cell types including gliomas. To evaluate a role of FasL in glioma-microglia interactions a FasL Interfering Protein (FIP) was developed.

Results: FasL and Fas mRNA and protein expression were detected in C6 glioma cells and primary astrocytes. In contrast to non-transformed astrocytes, rrFasL did not affect either death or proliferation of C6 glioma cells as shown by MTT metabolism test and flow cytometry cell cycle analysis. Glioma-conditioned medium (GCM) was able to activate microglial cells, which was associated with morphological alterations. Inhibition of this activation process abolished the promoting effect of microglia on tumor growth. We found that when Fas signaling was blocked in C6 glioma cells by FIP, there was no GCM-dependent microglia activation. In contrast, this effect was not observed, when FIP was added to GCM directly prior to microglia treatment. Blocking of the endogenous Fas signaling by FIP downregulated active JNK and p-38 MAPK but did not affect the level of phosphorylated ERK1/2. The decrease of phosphorylated JNK level was associated with downregulation of phospho-c-Jun, a component of AP-1 (Activator Protein – 1) transcription factor.

Conclusions: Our results suggest that Fas signaling in glioma cells is indespensible for gene expression and/or secretion of proteins involved in glioma-associated microglia activation. Moreover, FIP seems to be an effective agent for blocking FasL-Fas interactions.

Address List