isolated from freshly resected gliomas grade II, III and IV (each $n = 3$) using a modified magnetic bead protocol. Differentiation of cultured cells was assessed immunohistochemically using anti-GFAP, -NSE, -CD31, -CD105, -VE cadherin, Musashi-1 and AC133/1+2.

Results: CD133 positive cells could be detected in 7/10 gliomas WHO °II, 8/10 gliomas WHO°III and 9/10 GBM. These cells were found arranged in clusters, mostly associated to intratumoral vessels, rarely located diffusely within the tumor parenchyma. CD133 expression correlated with WHO grade: 1–5% of cells in gliomas WHO °II, 5–10% of cells in gliomas WHO °III, and 10–15% of cells in GBM stained positive for CD133. Western-blot analysis confirmed the correlation with tumor grade. CD133+ cells that have been isolated from specimens of all tumor grades stained positive for Musashi-1. Under different culture conditions, rapid proliferation of CD133+ cells occurred. After several passages, cells lost CD133 expression and became positive for GFAP, NSE or CD31/CD105/VE cadherin.

Conclusions: This study represents the first documentation for the presence of pluripotent, highly proliferative CD133+ CSC in low grade gliomas, which are able to differentiate into cells expressing glial, neuronal or endothelial markers. The presence of CSC in high grade gliomas could be confirmed, showing a higher proportion than in low grade gliomas. The role of these cells during stepwise glioma progression still has to be evaluated.

doi:10.1016/j.ejcsup.2006.04.092

P33. EXPRESSION OF ALPHA V BETA 3 INTEGRIN IN PATIENTS WITH HIGH AND LOW GRADE GLIOMA

O. Schnell[a], B. Krebs[b], E. Wagner[a], A.J. Beer[c], S.J. Grau[a], C. Goetz[a], H.A. Kretzschmar[b], R.H. Goldbrunner[a], J.C. Tonn[a]. [a]*Neurochirurgische Klinik, LMU München, Germany;* [b]*Zentrum für Neuropathologie und Prionforschung, LMU München, Germany;* [c]*Nuklearmedizinische Klinik, Klinikum rechts der Isar, TU München, Germany.*

Background: Here we show that the expression of αvβ3, a putative key player of angiogenesis in malignant glioma may be less pronounced in brain derived glial tumors as supposed by former studies and is not restricted to activated endothelial cells.

Methods: Cryosections of histopathologically confirmed high ($n = 25$) and low ($n = 10$) grade gliomas, were immunostained for detection of αvβ3-expression. Microvascular density of the samples was determined by co-staining with endothelial cell specific markers (CD31, CD105). Moreover Western blot analysis of consecutive cryosections was performed to further investigate the relative vascularisation and integrin expression in these tumors.

Results: Immunohistochemistry confirmed that high grade gliomas not only show a higher rate of αvβ3-positive proliferating endothelial cells but have a higher number of αvβ3-expressing tumor cells compared to low grade gliomas. Western blot analysis revealed that αvβ3-expression in malignant gliomas was higher as seen in low grade gliomas. Interestingly all glial tumors showed less integrin expression and less αvβ3-positive endothelial cells compared to solid non-CNS tumors. In return the rate of integrin-positive tumor cells was higher in all gliomas compared to the peripheral tumors.

Conclusion: Expression of αvβ3 integrin is lower in glial tumors compared with most peripheral solid tumors. In gliomas the fraction of tumor cells expressing αvβ3 is higher as in non-CNS tumors. Therefore the function of this integrin in brain derived tumors may not be restricted to angiogenesis alone and new anti-angiogenic drugs targeting this integrin may have control over the tumor cells themselves.

doi:10.1016/j.ejcsup.2006.04.093

P34. EXPRESSION OF LYMPHANGIOGENSIS RELATED VEGFR3 IN MALIGNANT GLIOMAS

S. Grau[a], F. Trillsch[b], N. Thon[a], O. Schnell[a], P. Nelson[b], J.-C. Tonn[a], R. Goldbrunner[a]. [a]*Department of Neurosurgery, LMU Munich University, Germany;* [b]*Department of Biochemistry, LMU Munich University, Germany.*

Background: Glioblastomas (WHO grade IV) are highly vascularised brain tumours. Targeting glioma angiogenesis several studies aim at the VEGF/VEGFR2 system, however, the presence and role of VEGFR3 in gliomas has not been investigated elaborately up to date. Here we show the high expression of VEGFR3 and its ligands in gliomas correlating with tumour grade.

Method: Human brain tumours WHO grade II ($n = 8$), grade IV ($n = 20$) and non neoplastic brain ($n = 3$) were investigated for expression of VEGFR-3, VEGF-C and VEGF-D on mRNA and protein level by use of real-time PCR, immunohistochemistry and Western blot analysis.

Results: Expression of VEGFR-3, VEGF-C and VEGF-D was very high in glioblastomas, scant in grade II gliomas and absent in non neoplastic brain. These findings were confirmed by Western blot. VEGFR-3 in glioblastomas was mainly present on tumor endothelium. VEGF-C and -D were expressed strongest in areas of high vessel density. On mRNA level, transcripts for all proteins were significantly elevated in glioblastomas compared to grade II gliomas and non neoplastic brain.

Conclusion: VEGFR3 expression correlates with tumour grade showing highest levels in glioblastomas. With also the receptor ligands VEGF-C and -D being strongly expressed, these findings reveal the presence of an alternative angiogenic signalling system in these tumours. This may influence the paradigm of glioma angiogenesis and may lead to more effective anti-angiogenic treatment strategies.

doi:10.1016/j.ejcsup.2006.04.094

P35. INTRATUMORAL PATTERNS OF CLONAL EVOLUTION IN MENINGIOMAS

R. Ketter[a], J. Rahnenführer[b], W. Henn[c], S. Wemmert[a], Y.J. Kim[d], T. Lengauer[b], K.D. Zang[b], W.I. Steudel[a], S. Urbschat[a]. [a]*Department of Neurosurgery, Saarland University, 66421 Homburg/Saar, Germany;* [b]*Max-Planck-Institute for Informatics, 66123 Saarbrücken, Germany;* [c]*Institute of Human Genetics, Saarland University, 66421 Homburg/Saar, Germany;* [d]*Department of Neuropathology, Saarland University, 66421 Homburg/Saar, Germany.*

Background: Meningiomas are usually benign tumors and cytogenetically well-characterized. Most tumors show either monosomy 22 or a diploid karyotype. Progression of meningiomas is correlated with increasing hypodiploidy and the loss of the short arm of chromosome 1. The aim of this study was to assess intratumoral patterns of clonal chromosomal evolution in order to identify tumor progression pathways and to analyze their correlation with time to recurrence.
Methods: From 1973 to 2004, 661 patients with complete tumor resections and cytogenetic characterization were followed up. We have developed oncogenetic trees mixture models for estimating the most likely order of cytogenetic aberrations.
Results: Overall, in 8.0% (53/661) of the tumors at least one recurrence was documented during the study. Our results showed a significant correlation between cytogenetic data and recurrence ($p < 0.001$), location ($p < 10^{-5}$) and WHO grade ($p < 10^{-15}$). The estimated model was used to assign a genetic progression score (GPS). The GPS of a tumor is a quantitative measure and allows precise assessment of genetic progression. We classified tumors in three groups with low genetic progression (GPS < 2), intermediate genetic progression ($2 \leqslant$ GPS < 6) and advanced genetic progression (GPS $\geqslant$ 6). The recurrence rate is 7.9% (27/343) in the low progression group, 4.0% (11/273) in the medium progression group, and 33.3% (15/45) in the high progression group.
Conclusion: Therefore, cytogenetic classification of meningiomas is a powerful tool to predict tumor recurrence and a valuable parameter for the postoperative management protocol.

doi:10.1016/j.ejcsup.2006.04.095

P36. THE CALCIUM BINDING PROTEINS S100A8 AND S100A9 AS NOVEL MARKERS FOR HUMAN PROSTATE CANCER

Alexander Hermani[a], Rainer Grobholz[b], Lutz Trojan[c], Peter Angel[d], Doris Mayer[a]. [a]*German Cancer Research Centre, Research Group Hormones and Signal Transduction, Heidelberg, Germany;* [b]*Department of Pathology, University Hospital Mannheim, Mannheim, Germany;* [c]*Department of Urology, University Hospital Mannheim, Mannheim, Germany;* [d]*German Cancer Research Centre, Division of Signal Transduction and Growth Control, Heidelberg, Germany.*

Background: S100 proteins comprise a family of calcium-modulated proteins that have recently been associated with epithelial tumours.
Methods: We examined the expression of two members of this family, S100A8 and S100A9 in human prostate adenocarcinomas by means of histochemical staining procedures. S100A9 was additionally analysed in patient serum using ELISA. Furthermore, the function of the two proteins was investigated in prostate derived cell lines using expression constructs and recombinant proteins.
Results: S100A8 and S100A9 were upregulated in prostatic intraepithelial neoplasia and preferentially in high-grade adenocarcinomas, whereas benign tissue was negative or showed weak expression of the proteins. Moreover, the analysis of S100A9 in patient serum revealed significantly elevated S100A9 serum levels in cancer patients compared to BPH (benign prostatic hyperplasia) patients or healthy individuals.[1]

In cell culture experiments S100A8 and S100A9 were identified as extracellular factors which induce MAP kinase and NF-κB signalling pathways and stimulate the migration of prostate epithelial cells.[2]
Conclusion: The data show that S100A8 and S100A9 are linked to the activation of important features of prostate cancer cells. Furthermore, S100A8 and S100A9 represent novel markers for prostate cancer, which may prove useful for future diagnostic and/or therapeutic approaches.

doi:10.1016/j.ejcsup.2006.04.096

P37. Gd-DOTA AND FLUOROPHORE SUBSTITUTED POLYAMINES AS INTRACELLULAR CONTRAST AGENTS FOR MAGNETIC RESONANCE AND FLUORESCENCE IMAGING OF TUMORS

Markus Wolf, William E. Hull, Ulrike Bauder-Wüst, Helmut Eskerski, Rüdiger Pipkorn, Michael Eisenhut. *German Cancer Research Center, Heidelberg, Germany.*

Background: Upregulation of polyamine transporters on the surface of tumor cells and the internalisation of biogenic polyamines by active transport processes may be exploited for the accumulation of millimolar quantities of reporter molecules.
Methods: Novel intracellular contrast agents for magnetic resonance imaging with high tumor uptake have been developed, based on Gd(III)-DOTA. Uptake of these agents into cultured tumor cell lines B16 (mouse melanoma), MH3924A (Morris hepatoma), A493 (kidney carinoma) and 3T3 NIH (mouse fibroblasts) was quantitated by ICP-MS. Furthermore fluorescence tagged polyamines were evaluated as optical imaging agents using confocal laser scanning microscopy to investigate uptake into B16 and MH3924A tumor cells.
Results: At 10 μM incubation with Gd(III)-DOTA-polyamine conjugates for 1 h, an uptake of 0.02–0.23 fmol/cell was achieved, corresponding to intracellular concentrations of 11–110 μM Gd. The cell uptake increased in the order A493 (0.02 fmol/cell) < 3T3NIH (0.03 fmol/cell) < B16 (0.05 fmol/cell) < MH3924A (0.23 fmol/cell). 0.017–0.17 fmol/cell internalized Gd is needed to achieve a detectable contrast enhancement via T_1-weighted MRI. Evidence for intracellular uptake of the fluorophore labeled polyamines in MH3924A and B16 tumor cells, investigated by confocal lase scanning microscopy, resulted in comparable uptake values as compared to the Gd(III)-DOTA derivatives. Initial in vivo studies showed that fluorophore labeled polyamines can be imaged in the tumor.
Conclusions: This study illustrates the potential of polyamine transporters which are upregulated in proliferating cells can be used for contrast agent enhanced MRI and optical imaging of tumors.

doi:10.1016/j.ejcsup.2006.04.097