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 II, 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.

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**P34. EXPRESSION OF LYMPHANGIOGENESIS RELATED VEGFR3 IN MALIGNANT GLIOMAS**


**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.


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**P35. INTRATUMORAL PATTERNS OF CLONAL EVOLUTION IN MENINGIOMAS**


**Method:** Human brain tumors 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.

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Conclusion: Meningiomas are usually benign tumors and cyto-
genetically well-characterized. Most tumors show either mono-
somy 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 intra-
tumoral patterns of clonal chromosomal evolution in order to
identify tumor progression pathways and to analyze their correla-
tion 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 esti-
mating 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 progres-
sion (GPS < 2), intermediate genetic progression (2 ≤ GPS < 6)
and advanced genetic progression (GPS ≥ 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 pro-
gression group.

Conclusion: Therefore, cytogenetic classification of meningio-
mas is a powerful tool to predict tumor recurrence and a valuable
parameter for the postoperative management protocol.

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

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Background: S100 proteins comprise a family of calcium-modu-
lated 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 intra-
epithelial neoplasia and preferentially in high-grade adenocarci-
nomas, 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 sig-
nalling pathways and stimulate the migration of prostate epithel-
ial cells.2

Conclusion: The data show that S100A8 and S100A9 are linked to
the activation of important features of prostate cancer cells. Fur-
thermore, S100A8 and S100A9 represent novel markers for pros-
tate cancer, which may prove useful for future diagnostic and/
or therapeutic approaches.

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

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Background: Uptregulation of polyamine transporters on the sur-
face of tumor cells and the internalisation of biogenic polyamines
by active transport processes may be exploited for the accumula-
tion of millimolar quantities of reporter molecules.

Methods: Novel intracellular contrast agents for magnetic reso-
nance 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 hep-
toma), A493 (kidney carcinoma) and 3T3 NIH (mouse fibroblasts)
was quantitated by ICP-MS. Furthermore fluorescence tagged
polyamines were evaluated as optical imaging agents using con-
focal laser scanning microscopy to investigate uptake into B16
and MH3924A tumor cells.

Results: At 10 μM incubation with Gd(III)-DOTA-polyamine con-
jugates 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 detect-
able contrast enhancement via T1-weighted MRI. Evidence for
intracellular uptake of the fluorophore labeled polyamines in
MH3924A and B16 tumor cells, investigated by confocal laser scan-
ning microscopy, resulted in comparable uptake values as com-
pared 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.

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