

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.

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

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Background: Here we show that the expression of $\alpha v\beta 3$, 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 $\alpha v\beta 3$ -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 $\alpha v\beta 3$ -positive proliferating endothelial cells but have a higher number of $\alpha v\beta 3$ -expressing tumor cells compared to low grade gliomas. Western blot analysis revealed that $\alpha v\beta 3$ -expression in malignant gliomas was higher as seen in low grade gliomas. Interestingly all glial tumors showed less integrin expression and less $\alpha v\beta 3$ -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 $\alpha v\beta 3$ integrin is lower in glial tumors compared with most peripheral solid tumors. In gliomas the fraction of tumor cells expressing $\alpha v\beta 3$ 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.

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P34. EXPRESSION OF LYMPHANGIOGENESIS RELATED VEGFR3 IN MALIGNANT GLIOMAS

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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

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