P30. PROGNOSTIC VALUE OF PROSTATE CIRCULATING CELLS DETECTION IN PROSTATE CANCER PATIENTS: A PROSPECTIVE STUDY

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Background: During the natural history of prostate cancer, bloodstream prostate cell dissemination occurs, even in clinically localised prostate cancer patients. Additionally, prostate manipulation during radical prostatectomy induces hematogenous prostatic cell dissemination. Both may play an adverse role on the natural course of the disease. Blood-borne circulating prostate cell detection might be helpful to better classify patients and predict recurrence.
Methods: To evaluate cancer-cell seeding impact upon patient recurrence free survival, 111 prostate cancer patients were prospectively enrolled and followed up. Prostate cell spread was assessed by PSMA-based RT-PCR. Positive recurrence was defined by two consecutive serum PSA values $\geqslant 0.2$ ng/L.
Results: Forty-one patients presented blood prostate cell shedding preoperatively and intraoperatively (group I). Of the 70 preoperatively negative patients, 38 (54%) remained negative (group II) and 32 (46%) became intraoperatively positive (group III). Median biological and clinical recurrence-free-time was far shorter in group I (36.2 months, $p < 0.0001$) than in group II (69.6 months) but did not significantly differ in groups II and III (69.6 months versus 65.0).
Conclusions: Such 5-year follow-up data show that preoperative circulating prostate cells are an independent prognosis factor of recurrence. Moreover, tumor handling induces cancer-cell seeding but this surgical blood dissemination does not accelerate cancer evolution.

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P31. SURVIVIN mRNA LEVELS IN PERIPHERAL BLOOD FROM PATIENTS WITH ESOPHAGEAL CANCER DECREASE SIGNIFICANTLY FOLLOWING SURGICAL RESECTION AND ARE INFLUENCED BY NEOADJUVANT CHEMORADIATION

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Background: Survivin is an inhibitor of apoptosis and specifically expressed in several human cancers. The purpose of this study was to evaluate if there is a difference in the level of survivin mRNA expression between different histological types and stages of esophageal cancer. Furthermore we evaluated if a potential postresectional decrease in survivin mRNA expression might be used to verify complete surgical resection and if neoadjuvant chemoradiation would influence these results.
Methods: Blood samples were obtained from 47 patients who were scheduled for surgical resection of an esophageal carcinoma. 21 (44.7%) patients had squamous cell carcinomas and 26 (55.3%) adenocarcinomas. In 21 (44.7%) patients neoadjuvant chemoradiation was perforemd for locally advanced disease. Whole blood was drawn one day preoperatively and 10 days post resection in all patients. The tumor cells were enriched from whole blood by density gradient centrifugation (OncoQuick®, Hexal, Frickenhausen) and total cellular RNA was extracted. Direct quantitative real-time reverse transcriptase PCR (RT-PCR, TaqMan™) assays were performed in triplicates to determine survivin mRNA expression levels.
Results: Survivin mRNA expression in peripheral blood was detected in 35/47 patients (74.5%). There were no significant differences in preoperative mRNA levels between squamous cell carcinomas and adenocarcinomas. Postoperative survivin levels were significantly lower than preoperative levels in 41.2% of resected patients. Postoperative levels were lower than preoperative levels in 52.9% of patients with adenocarcinomas and 29.4% of patients with squamous cell carcinomas. Patients who received neoadjuvant chemoradiation had significantly lower survivin mRNA levels postoperatively in 66.7% compared to 27.3% of patients following primary resection (Wilcoxon test: $p < 0{,}027$). In patients receiving neoadjuvant chemoradiation postoperative mRNA expression levdels were detected at 83.3% of patients with adenocarcinomas compared to only 36.4% with squamous cell cancer.
Conclusions: Our results demonstrate that direct real-time quantitative RT-PCR analysis of survivin mRNA expression without prior nested PCR is technically feasible and reliable following tumor cell enrichment from whole blood samples in patients with esophageal cancer. Survivin levels were significantly reduced following surgical resections and might become a molecular marker for completeness of resection (molecular R0 marker). Survivin levels particularly decreased in adenocarcinomas following neoadjuvant chemoradiation and surgical resection.

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P32. CD133 POSITIVE "CANCER STEM CELLS" IN GLIOMAS OF DIFFERENT GRADES

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Background: Within glioblastoma multiforme (GBM) specimens, a subpopulation of CD133 positive cells with ability for self renewal and tumor generation could be isolated suggesting the presence of "cancer stem cells" (CSC) in GBM. Aim of this study was (i) to assess the presence of CD133+ cells in gliomas grade II, III and IV and (ii) to investigate the differentiation potential of these cells.
Methods: Samples of WHO °II (10), WHO III (10) and WHO °IV (10) gliomas were investigated immunohistochemically using the antibody AC133/1+2, which binds to two different epitopes of CD133, and Musashi-1, an established stem cell marker. Additionally, CD133 expression was assessed by ELISA. CD133+ cells were

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 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.

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P34. EXPRESSION OF LYMPHANGIOGENSIS 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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