1 pt. CK-20 before manipulation (I)

Discussion: The results indicate that at least in some patients tumor cell dissemination occurs during surgery. Further data is required to determine the diagnostic sensitivity and specifity of tumor cell detection using RNA- and DNA markers, resp., and to estimate the prognostic and clinical relevance of intraoperative tumor cell dissemination.

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Human glioma cells: genetic alterations and radiation and temozolomide sensitivity

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Purpose: Determination of the genetic profile of early passage cell cultures derived from human gliomas and established glioma cell lines and correlation with their in vitro radiation- and temozolomide sensitivity.

Materials and Methods: Cell cultures of 6 patients were processed from fresh brain tumour specimens. Also the glioma cell lines Gli 6 and D 384 were used. Genetic characterisation included determination of mutational and loss of heterozygosity (LOH) status of TP 53, LOH of chromosome 10 status and Epidermal Growth Factor Receptor gene (EGFR) amplification. Irradiation of cells ranged from 0 to 6Gy (RT), or cells were treated with TMZ by incubation for 3h or 24h in 10 ZM TMZ-containing culture medium (3hTMZ; 24hTMZ). For combined treatment, cells were irradiated immediately following 3hTMZ and 24TMZ. Cell survival was determined by clonogenic assay and survival curves were generated. Surviving Fractions after 2Gy (SF2) and 4Gy (SF4) were used as radiosensitivity parameters.

Results: The genetic profile of Gli 6 shows LOH but no mutation of TP 53, complete LOH 10 but no EGFR amplification. In the genetic characterisation of VU 15 a mutation and LOH of TP 53, but no LOH 10 and EGFR amplification were diagnosed. The VU 19 cell culture showed incomplete LOH 10 but no other genetic aberrations. In the VU 24 cell culture an incomplete LOH 10 and EGFR amplification, but no TP 53 alteration was seen. The other cell cultures and

D 384 cell line showed no genetic aberrations. Surviving fractions after 3hTMZ ranged from 0.19 to 0.91 (mean: 0.65) and from 0.16 to 0.72 (mean: 0.49) after 24hTMZ. SF2/SF4 values after RT alone ranged from 0.47/0.24 to 0.90/0.54 (mean: 0.73/0.36) from 0.36/0.15 to 0.75/0.51 (mean: 0.60/0.30)after 3hTMZ RT and from 0.35/0.15 to 0.68/0.45 (mean: 0.54/0.26) after 24hTMZ RT. Two cell cutures (VU 15, VU 20) showed a reduction in cell survival after 3hTMZ to 0.19 and 0.52 respectively while the other cell cultures and established cell lines showed surviving fractions of >0.55 after 3hTMZ.

Conclusions: Glioma cells show a low radiosensitivity. Combination of RT with TMZ decreased the SF2- and SF4 values, with a trend towards synergistic effect in TMZ sensitive cells and additive effect in less TMZ sensitive cells. Alterations in cell regulatory genes are frequently found and, together with the data on radiation and TMZ sensitivity, this information might be used for individualized therapy of glioma patients.

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Helix pomatia agglutinin (HPA) binding: an independent prognostic factor in resected adenocarcinomas of the lung

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Introduction: The incidence of adenocarcinoma of the lung rises worldwide, unfortunately no significant prognostic marker beyond the classical TNM staging exists to stratify the patients for appropriate therapy. A panel of lectins (Helix pomatia agglutinin (HPA), Phaseolus vulgaris agglutinin (PHA-L), Ulex europaeus agglutinin (UEA-1), Maackia amurensis agglutinin (MAA), Sambucus nigra agglutinin (SNA-I)) with different carbohydrate specificities were tested for their prognostic relevance.

Patients and Methods: Paraffin wax sections of 93 patients (pts) with adenocarcinomas of the lung who had undergone surgery between 1990 and 1995 were investigated by lectin histochemistry. All pts were followed up systematically for a minimum of up to five years.

Results: 63 male/30 female; median age 59 years [range 27-81]; 72 pts stage I/II, 19 pts stage IIIA, 1 pts stage IIIB, 1 pts stage IV disease. The overall 5-year survival rate was 49.5%. Distant metastases or local relapse were diagnosed in 49 patients (53%). 9 tumours were classified as HPA-negative, and 83 as HPA-positive. Pts with HPA-positive tumours had a significantly poorer survival than pts with HPA-negative tumours (p=0.015, log rank test). All patients with HPA-negative tumours survived 5 years. Next to HPA also binding of PHA-L (p=0.017, log rank test) and UEA-I (p=0.022, log rank test) to adenocarcinoma cells were prognostic indicators for overall survival, whereas MAA and SNA-I binding had no prognostic significance. In multivariate Cox regressions analysis next to stage (stage II: p=0001, standard error 0.39, risk ratio 6.00; stage III/IV: p=0001, standard error 0.39, risk ratio 8.35) and gender (p=0004, standard error 0.37, risk ratio 3.65) only HPA (p=0.04, standard error 1.06, risk ratio 8.75) was an significant independent prognostic factor on survival.

Conclusion: HPA binding was the primary marker-based predictor of prognosis in our patient population and allows to stratify patients with adenocarcinomas of the lung into a low and a high risk group.

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The response of advanced pancreatic cancer (APC) to gemcitabine monochemotherapy in relation to the expression of proliferation markers, oncogenes Her-2, Bcl-2, C-myc and p53 antioncogene. A retrospective clinico-pathological study

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Background: Gemcitabine(G),a nucleoside analogue,is accepted as palliative treatment in APC. The cytotoxic effect of G is mediated by the DNA damage in proliferating cells, followed by an induction of apoptosis. The objective of this study was to determine, whether some immunohistochemical analysis may be useful in predicting G efficacy.

Material and methods: An immunohistochemical analysis of archived, formalin fixed, paraffin embedded tumor samples was performed in 25 patients(pts) with histologically confirmed measurable APC, treated with G. The expression of cell cycle markers Ki67, Cyclin A and Cyclin B1 has been scored as percentage of positive cells, while the Her-2, Bcl-2, C-myc and p53 expression has been rated as none, moderate or strong. The pts have been followed clinically and their objective response rate(ORR) and overall survival(OS) were recorded and compared to the results of immunohistochemistry. Results: There was no relation between the pts OS and the tumor proliferation reflected by the expression of the above mentioned markers. The overexpression of Her-2 and Bcl-2 oncogenes in tumor cells was a rare finding (one case each), a substantial expression of C-myc was recorded in 3/25 cases only. A strong expression of p53 antioncogene in tumor cells was noted in 8/25 cases, while no staining appeared in 3/25 cases. In 14/25 cases the p53 staining was of a moderate level ranging from 5-80% of tumour cell nuclei. If the pts from the later group were compared to those ones with tumor exhibiting none or very strong p53 expression, their average OS was longer (6.5 vs. 4.2 months, p=0.03-t-test) and the survival curves differed significantly (p=0.04 - Mantel-Cox).

Conclusions: The pts suffering from APC with moderate p53 expression (corresponding to the partial maintenance of p53 function,i.e. moderate level of staining) respond better to the G chemotherapy and have longer OS comparing to the pts with carcinoma with no staining (corresponding to p53 deletion or posttranslational modification of target epitope) as well as comparing to the pts with carcinoma with strong p53 expression (corresponding to the stabilization of non-functional p53 due to the missense mutation). These data suggest that detailed evaluation of p53 status might be of interest in the attempt to predict the pts response to the G chemotherapy of APC.

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