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Microcarriers enhance the efficacy of PA317/HSV-1 tk gene therapy in 9L rat glioma model

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Glioblastoma accounts for 1/3 of primary brain tumors. Despite the use of combinations of therapeutic modalities (surgery, chemotherapy, radiation), prognosis is poor, with a median survival of one year and a 9% five-year survival rate. Protocols utilizing viral vectors to deliver a gene for the treatment of malignant gliomas have been developed, and some are in clinical trials. Retrovirus carrying herpes simplex virus tk gene in combination with ganciclovir represents one approach. To improve productivity of retrovirus in vivo, intratumoral injection of retrovirus-producing cells has been used. This approach relies on survival of virus-producing cells in the tumor following injection. Attachment of cells to microcarriers (MC) has previously been shown to increase their survival and function after implantation in Parkinson's disease studies. We therefore tested the effects of MC on HSV-1 tk/ganciclovir gene therapy in the 9L rat glioma model, using PA317 cells carrying a retroviral vector expressing tk gene. Young adult Wistar rats received intrastriatal injections of 5×10^4 9L glioma cells. On day 10 after tumor implantation, 5ul of PBS, PA317 cells (1.8×10^4), or PA317 cells attached to MC (1.8×10^4 cells plus 250ug MC) were injected intratumorally to randomly-divided animals (n = 8 per group). Starting 15 days following tumor implantation, all animals received 25mg/kg ganciclovir i.p. daily for 10 days. Results show that treatment with PA317 cells and ganciclovir had a weak therapeutic effect compared to PBS control group, showing a slightly extended mean survival time (29±1days vs 25±1 days, p=0.0498). MC-attached PA317 cells significantly increased the mean survival time from 29±1 days for PA317 cells only to 48±5 days for MC/PA317 cells (p=0.0052). In addition, approximately 50% of animals treated with MC/PA317 cells survived over 60 days tumor-free while 100% mortality was observed in PBS and PA317 only groups. These results suggest that attaching tk-expressing cells to MC significantly enhanced efficacy of gene therapy for intracranial glioma model, and that MC may represent a general method for enhancing gene therapy to the brain. We are currently using immunocytochemistry to measure life span of implanted PA317 cells by pre-labeling with BrdU, and examining immune response of host animals with or without MC. Possible mechanisms of the enhanced effect of cells attached to microcarriers will be discussed.

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The dose-volume interaction in adult supratentorial low-grade glioma: higher radiation dose is beneficial among patients with partial resection

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Purpose: To test the hypothesis that adult patients with supratentorial low-grade glioma (LGG) and partial surgical resection (PR, ≤ 50% resected) benefit from higher doses of radiation.

Methods: Patients treated with immediate post-operative radiation for WHO grade I-II LGG at the University of Western Ontario between 1979-2001 were studied. Distribution of patient characteristics was compared among those receiving PR and those obtaining subtotal/total resection (STR, > 50% resected). Age > 40 yrs, gender, duration of symptoms > 30 days, seizures at presentation, Karnofsky performance status (KPS) < 70, astrocytoma pathology (AS), and radiation dose ≤ 50 Gy were compared. A Cox regression model was constructed among patients with partial resection to test the influence of radiation dose. Subsequently, a similar model was constructed of the entire patient sample.

Results: One hundred and seven patients were analyzed. Patients who had PR were not significantly different from those with STR when age, gender, symptom duration, seizures, KPS, pathology, or median radiation dose were considered. Among the PR group, 69% had AS histology (N=41), 39% were > 40 years age (N=23) and 29% received ≤ 50 Gy (N=17). Seven patients in the PR group received doses < 42 Gy. Median survival (MST) of patients who received doses ≤ 50 Gy and PR (N=19) was 16.5 months while those who received doses > 50 Gy with PR had a MST of 109.2 months. A Cox regression model of patients with PR was highly significant with radiation dose influencing survival (OS) after controlling for age and histology (p=0.005). Subsequent modelling of the entire group (PR+STR) was completed. The interaction of radiation dose and residual tumour volume was tested after controlling for age and histology. The regression model was highly significant for both OS and PFS (p=0.013 and p=0.003 respectively). The model remained significant after patients receiving doses < 42 Gy (N=7) were excluded (OS p=0.024, PFS p=0.001).

Conclusions: Outcome for adult LGG is highly dependent on post-surgical tumour volume and radiation dose. Patients with partial resection should be considered for higher radiation dose schedules (> 50 Gy). Subgroup analysis of EORTC and RTOG- NCCTG-ECOG dose-response trials seems warranted.

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Telomere length as a prognostic marker in glioblastomas

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Although glioblastomas multiforme are amongst the malignant human tumours with poor prognosis considerable differences do occur between the length of survival for patients with this disease. This suggests the presence of intrinsic factors that may influence outcome. Unfortunately at present the molecular basis of this variation is not known and there are no markers for identifying the 10 to 15% who are deemed 'long term survivors'. Telomeres are essential structures at the ends of chromosomes which are eroded during cell division. Telomere shortening is viewed as a tumour suppressor mechanism which has to be overcome if cells are to become immortal. The objective of this study was to determine the effect of telomere length and maintenance on survival in patients with high grade gliomas. We studied glioblastomas from 30 patients for telomere length and telomerase activity. We found a subset of nine glioblastomas with very long telomeres, a hallmark of the Alternative Lengthening of Telomeres or ALT phenotype while 21 patients had non-ALT tumours. Median survival time for all 30 patients was 357 days (95% CI 244 - 470). Patients with ALT gliomas had a mean post operative survival of 647 days compared to 327 days for patients with 'normal' telomeres treated similarly (p=0.05). 67% of patients with ALT glioblastomas are 'long term'survivors. Patients with telomerase positive tumours had a significantly shorter survival time than those with telomerase negative tumours (257 vs. 411 days p=0.05). We suggest that telomere length and maintenance mechanisms may have a bearing on prognosis for patients with glioblastomas.

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Occupational risk factors for brain tumours in adults. Results from the international brain tumour case- control study

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Purpose: Risk factors for brain tumours have been discussed for various occupational exposures frequently with inconsistent results. The presented study provides the opportunity to further evaluate the etiologic role of occupation for glioma and meningioma.

Methods: A population-based case control study was performed in the late 1980's in eight study centres in Australia (Adelaide, Melbourne), Canada (Winnipeg, Toronto), Europe (France, Germany, Sweden) and USA (Los Angeles). 330 meningioma and 1178 glioma cases, all histologically confirmed, were included. Controls have been individually matched by 5-year age group, gender and region (1123 controls for meningioma and 1987 controls for glioma). Lifetime occupational history was obtained from all cases and controls. Occupational activities were coded by means of ISCO and subsequently grouped into 16 occupational categories. Six of these categories were examined, because of a priori determined hypothesis, initiated by respective literature: chemical, metal, electrical, agricultural, construction and transport. For each tumour type a pooled analysis was performed separately, using conditional logistic regression to estimate relative risks (RR) and 95% confidence intervals (CI) (adjusted for years of education).

Results: No increased occupational risk factors could be identified for gliomas. For meningiomas, however, increased risks were found for employment in the categories transport (RR=1.87, 95%CI 1.04-3.37) and construction (RR=1.94, 95%CI 1.17-3.20). For gliomas the risks were slightly decreased for involvement in the chemical (RR=0.73, 95%CI 0.50-1.06), the electrical (RR=0.87, 95%CI 0.64-1.19) and the agricultural group (RR=0.86, 95%CI 0.66-1.12). No other risk factors could be identified.

Conclusion: This international multicenter case-control study is one of the largest brain tumour study in adults. Occupational risk factors could only identified for meningiomas but not for gliomas. We were not able to confirm the hypothesis of risk increase for employment in chemical, metal, agricultural or electrical industries.

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Tumour class prediction and discovery by microarray-based DNA methylation analysis

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Class prediction is of crucial importance for most therapeutic decisions in cancer. Recent studies have shown that classification of cancers can be achieved by mRNA expression monitoring. However, due to difficulties with handling mRNA samples, expression analysis is not widely used for large-scale analyses or clinical settings. Here we present a novel, more robust approach to cancer classification based on analysis of characteristic DNA methylation patterns. Information on methylation status is obtained for many sites in parallel using a novel DNA-based microarray technology. Methylation patterns are then presented to a learning algorithm to perform class prediction. In addition, hierarchical clustering methods can be used for class discovery. Our results demonstrate that analysis of methylation patterns combined with supervised and unsupervised learning techniques constitutes a powerful tool to classify human cancers.

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Application of genomic real-time PCR to characterise 11q deletions in mantle cell lymphoma and chronic lymphocytic leukaemia

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Purpose: Chromosome 11q22-23 is frequently deleted in human solid and lymphoid neoplasms, including mantle cell lymphoma (MCL) and chronic lymphocytic leukaemia (CLL). Candidate cancer genes have been mapped to this region. Deleted regions vary in size and can also be discontinuous. It is assumed that genes within these regions could affect the function the malignant cells. Although usually performed to quantify gene expression, real-time PCR can be applied to genomic DNA where it is able to discriminate between one and two copies of a target sequence. In order to characterise the pattern of deletions in lymphoproliferative disorders at a molecular level, a chromosome 11q22-ter PAC/BAC contig was initially constructed and real-time PCR primers were designed from sequences within this contig to potentially map the extent of deleted regions.

Methods: BAC and PAC clones were identified and mapped by screening of different human genomic libraries by PCR, filter hybridisation and database searches, using sequence-tagged sequences (STSs), expressed sequence tags (ESTs) or gene sequences known to map to the region of interest. The PAC and BAC end sequences were then used as new STSs for database analysis or to design PCR primers, after filtering the repetitive elements using the BLAST software. Real-time PCR primers were designed with the assistance of the ABI Primer Express software. DNA samples from MCL and CLL samples were analysed in triplicate, using the ABI SYBR Green PCR Master Mix and run on an ABI Prism 7700.

Results: A first group of 31 MCL, classic variant, and 25 CLL cases were analysed. Chromosome 11q22-23 deletion was detected in 6 (19%) MCL and in 11 (44%) CLL cases. Cases with deletions underwent further investigation with different primers to succesfully characterise the detailed extent of the deletion.

Conclusion: The combination of data originating from the BAC/PAC assembled contig together with those obtained by genomic real-time PCR on tumour samples provides a new promising tool to understand the pathogenesis of CLL and MCL. Analysis of further MCL and CLL cases is under way. Supported by the Leukemia Research Fund, the Krebsforschung Schweiz, and the Swiss Group for Clinical Research (SAKK).

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Alterations of CDKN2A, p53 and related cell cycle regulatory genes in esophageal squamous cell carcinoma

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Purpose: Alterations of the tumor suppressor genes CDKN2A and p53 represent essential steps in the tumorigenic process in a variety of cancer types. We investigated these genes and the related cell cycle regulators p14ARF, CDKN2B, CDKN2C, CDK4, and p53R2 for alterations in 21 cases of esophageal squamous cell carcinoma from China.

Methods: Mutational analysis was performed by SSCP-sequencing. The methylation status was analysed for the CDKN2A and p14ARF genes by methylation-specific PCR. LOH was analysed for five microsatellite markers flanking the INK4-ARF locus and one marker at the p53 locus.

Results: Frameshift or premature stop codon mutations in the CDKN2A gene were identified in six cases and hypermethylation of the promoter region in four cases. The correlation between mutation or methylation in the CDKN2A gene and LOH at the INK4-ARF locus was significant (Fischer exact test, P=0.001). A CDK4 mutation was detected in one case which also had a mutation in the CDKN2A gene, suggesting lack of function for one of the mutations. A high frequency of methylation was detected in the promoter region of the p14ARF gene (52%). Mutations in the p53 gene were detected in 14 cases, of which one case was found to have two mutations located in exon 5.

Conclusion: The study provides clear evidence to the involvement of the CDKN2A and p53 genes in esophageal cancer. A high prevalence of biallelic inactivation of the CDKN2A gene was detected in the esophageal cancer cases. Furthermore, a role of the p14ARF gene is indicated by the frequent methylation of its promoter, although no mutations were observed in exon 1b. Other cell cycle regulators studied, CDKN2B, CDKN2C, CDK4 and p53R2, did not show important roles in the esophageal cancer cases.

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Evidence for adeno-associated virus-induced cellular factor(s) in transgenic mice, mediating inhibition of the human papillomavirus type 18 promoter

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The cervix uteri has been found to be frequently co-infected with both, human papillomaviruses (HPV) and the helper virus-dependent adeno-associated viruses (AAV). Sero-epidemiological data suggest that AAV infection could inhibit cervical cancer caused by "high risk" types of HPV. In vitro, infection with AAV type 2 (AAV-2) or transfection of AAV-2 early (rep) genes has been shown to inhibit transformation by HPV. To analyze effects of AAV on HPV in vivo we studied the influence of AAV-2 infection on the promoter of "high risk" HPV type 18 (HPV-18) in mice, transgenic for sequences of the Upstream Regulatory Region (URR) of HPV-18 controlling transcription of the reporter gene, lacZ. Transgenic animals (or tongue cells thereof, explanted in culture) were treated with dexamethasone (DEX) to induce the HPV-18 promoter. Simultaneously they were (i) infected with AAV, (ii) inoculated with AAV virus-like particles (VLPs; empty capsids) or (iii) mock infected. Inoculation with AAV-2 or VLPs inhibited activation of the HPV-18 promoter. In vitro, in BHK cells transfected with the HPV-18-lacZ construct, tissue extracts from AAV-infected animals suppressed the HPV-18 URR to a similar extent as AAV-infection did. Down-regulation of the HPV-18 promoter was less efficient with extracts from animals inoculated with VLPs,