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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78 POSTER DISCUSSION

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.

79 POSTER DISCUSSION

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).

80 POSTER DISCUSSION

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.

81 POSTER DISCUSSION

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,