other three were pooled together. As the initial material isolated from the core biopsies was not sufficient for microarray analysis, we performed an amplification procedure using a modified Eberwine protocol. A complementary DNA microarray consisting of 6,000 human genes was used. Comparison of the array results from core biopsies (amplified RNA) and surgical specimens (non-amplified RNA) showed maintenance of the expression profile and concordance in identifying outliers in the range of 58%. This finding compares with a 48–77% concordance observed among three different samples of the same excisional biopsy using total RNA. The level of concordance was higher when an amplified core biopsy was compared with an amplified excisional biopsy: 64% for the Ewing sarcoma and 83% for the neuroblastoma. Pooling the core biopsies did not improve the concordance with surgical biopsies. Gene expression profiles obtained from microarray analysis differentiated Ewing sarcoma from neuroblastoma with both core and surgical biopsies as starting material. Our results suggest that core biopsy samples can be used as acceptable and reliable material for the determination of tumor genetic profiles.

Stenman, Göran K.D.

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Fusion of the NH2-terminal domain of the bHLH protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9;15)(q22;q21)

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Extraskeletal myxoid chondrosarcomas (EMC) are characterized by recurrent t(9;22) or t(9;17) translocations resulting in fusions of the NH2-terminal transactivation domains of EWS or TAF2N to the entire TEC protein. We report here an EMC with a novel translocation t(9;15)(q22;q21) and a third type of TEC containing fusion gene. The chimeric transcript encodes a protein in which the first 108 amino acids of the NH2-terminus of the basic helix-loop-helix (bHLH) protein TCF12 is linked to the entire TEC protein. The translocation separates the NH2-terminal domain of TCF12 from the bHLH domain as well as from a potential leucine zipper domain located immediately downstream of the breakpoint. These results demonstrate that the NH2-terminal transactivation domains of EWS or TAF2N are not essential for the oncogenic properties of fusion proteins in EMC, and that EWS or TAF2N may be replaced by a similar domain from a bHLH protein that presumably endows the fusion protein with similar functions.

Stephan, D.A.

[39]

Identification of expression changes of prognostic and therapeutic value in metastasizing medulloblastoma

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Medulloblastoma is a highly malignant cerebellar tumor of children and, less frequently, of adults, with a tendency to early recurrence and dissemination. Despite recent advances in patient survival, the quality of life for survivors is poor due to neurocognitive, neuroendocrine, and hearing deficits as a direct result of the

whole brain and spine radiation required for prevention of metastatic disease. Using global expression profiling, we identified genes prognostic of tumor metastasis that may also serve as new therapeutic targets. In the first phase of the study, an expression scan was done using a pooling approach, assuming that genetic heterogeneity would not be a significant confounding factor. We isolated total RNA from six metastasizing and six non-metastatic tumors, made biotinylated cRNA, and pooled samples from the two clinical groups in equimolar amounts. Gene expression profiles were generated using Affymetrix HuGeneFL Gene Chips (5600 genes). 266 genes showed differential expression greater than two-fold. The 58 genes expressed at least three-fold more or less between groups were selected for further analysis. 21/30 genes with increased expression in metastatic tumors have been implicated in promoting: invasion/metastasis (14), cell growth (4), and angiogenesis (3). 12/28 genes with decreased expression in metastatic tumors have been implicated in inhibiting: invasion/metastasis (5), cell differentiation (6), and cell growth (1). Expression of each gene is being characterized via tissue array immunostaining with 140+ paraffin embedded tumors (45/58 genes have available antibodies). In order to evaluate the efficacy of pooling clinical samples and to identify further candidates in the second phase of the study, individual oligonucleotide arrays using 80+ frozen tumors are being performed, and correlated with clinical and pathological data. This study has identified historically valid candidate genes involved in metastatic medulloblastoma, and has shown that a pooling strategy can be utilized effectively to identify candidate genes without complex statistical analysis, as long as validation studies are part of the study design. In addition, we show pooling may be an alternative strategy to identify relevant 'candidate' predictor genes when only small numbers of clinical samples are available.

Sulman, Erik P.

[40]

Identification of candidate tumor suppressor genes in meningioma by regional expression profiling

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Meningioma is a common and frequently recurrent central nervous system tumor. Hemizygous deletion of the short arm of chromosome 1 (1p) is second only to monosomy of chromosome 22 as the most frequently observed chromosomal alteration in these tumors. We previously identified a smallest region of overlapping deletion (SRO) spanning 1.5 centimorgans on 1p32; 1p allelic loss was predictive of tumor recurrence and correlated with loss of chromosome 22 and mutation of the *NF2* tumor suppressor gene. Deletion of this region has also been observed in oligodendroglioma and neuroblastoma. To identify transcripts in the SRO, we constructed a physical map of the region. We have collected 80 large-insert clones assembled into 4 contigs spanning approximately 3 megabases. Preliminary analysis of clone sequence has identified several previously unmapped genes in the SRO. A total of 78 unique transcripts have been localized to the region. We identified additional genes of interest through analysis of the expression differences between a normal meninges cell line, LTAg2B, and a meningioma cell line, KT21MG1, using Atlas Cancer Gene blots. To identify candidate tumor suppressor genes we are profiling the expression of all identified transcripts as well as other genes previously proposed to play a role in meningioma tumorigenesis, such as *NF2*, *LIF* and *ADTBI*, using a custom-made, low-resolution, glass slide complementary DNA microarray. Ultimately we plan to obtain a panel of candidate transcripts exhibiting tumor-specific changes in expression that can be examined further for genomic rearrangements or for mutations in tumors with characteristic deletions in the SRO.