HACE1 SHOWS TUMOR SUPPRESSOR ACTIVITY IN WILMS' TUMOR AND OTHER PEDIATRIC MALIGNANCIES

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We have recently observed that HACE1 (HECT domain and Ankyrin repeat Containing E3 ubiquitin-protein ligase 1) levels are lower in Wilms' tumor compared with normal kidney and that low expression is correlated with methylation at two CpG islands upstream of the HACE1 gene. In addition, despite widespread expression throughout normal tissues we have observed consistently low expression in a number of pediatric small round cell tumor lines. This leads us to hypothesize that HACE1 functions as a tumor suppressor gene. To explore this role of HACE1, we have established a retroviral model of stable HACE1 overexpression. We have thereby shown that Wilms' tumor and some other small round cell tumor lines, including neuroblastoma-derived cell lines, have less tumor-forming potential, (with reduced in vitro colony formation in soft agar) when over-expressing HACE1 compared with empty vector or a non-functional HACE1 mutant. In vivo tumor formation in nude CD-1 mice injected with a Wilms tumor cell line overexpressing HACE1 compared with empty vector control is also highly attenuated. Furthermore, we have identified a number of small interfering RNA (siRNA) sequences to functionally knock-down HACE1 expression. Using these siRNAs in a stable lentiviral system we have reduced HACE1 expression by as much as 80% in a number of cell lines in order to recreate a tumor environment. In HEK (human embryonic kidney) 293 cells with knocked down HACE1 expression, this resulted an increase in the number and size soft agar colonies. From these data, we conclude that HACE1 does indeed act as a tumor suppressor gene. Future work will involve identifying components of the Hace1 pathway including potential therapeutic targets.

DATABASE INTEGRATION AND DATA VISUALIZATION FOR BIOMARKER DETECTION IN PEDIATRIC CANCER

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Concerted efforts including improvements in adjuvant chemotherapy, surgery and radiation therapy have dramatically improved the prognosis of childhood cancer. One current focus is to reduce morbidity of treatment for low risk patients and reserve more intensive treatments for high risk patients. Therefore, a major research effort is to find biomarkers that could be used to predict patient response. Recent advances in high throughput technologies such as gene expression profiling and proteomics are dramatically changing study designs for the treatment of breast cancer. The vast explosion of the amount of information generated requires new means to support data management that allows for the integration of clinical data with data from various other sources. Moreover, to take full advantage of these technologies, radically different computational methodologies need to be used. As an example, we are pursuing high throughput serum biomarker discovery for Wilms tumor and other pediatric solid tumors by first using data available in databases from genomic analyses and from databanks of protein characteristics to establish an in silico biomarker profile prior to the wet lab experimentation. Data was selected from all of the available databases based on the quality of the data sources, the data coverage, and the standardization of data elements. The retrieval protocol was integrated into the System for the Integration of Bioinformatics Services (SIBIOS), which was developed at Indiana University. Advanced visualization techniques were developed to access, manage and make sense of large-scale complex datasets. These techniques allow us to get a global view of available data, to visually compare and correlate data from different sources, and to quickly filter out and select entities and associations on demand. The data integration protocols and visualization tools developed are applicable for research projects on a wide range of cancers for which serum/plasma and tumor samples are currently being collected.

INTEGRATED GENOMIC AND EXPRESSION PROFILING OF ANAPLASTIC WILMS TUMOUR

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The cyclin-dependent kinase (CDK) inhibitor p16INK4a, housed at 9p21, is a key mediator involved in cell cycle regulation. Disruption of this locus has been observed in many types of human cancer. Previous studies have identified decreased expression to be associated with increasing tumour stage in favorable histology (FH) Wilms tumours, with approximately 20% of samples showing loss of expression. Analysis of 121 Wilms tumours by microarray-based comparative genomic hybridisation (aCGH) has highlighted deletions involving the short arm of chromosome 9. Overall, 21/121 (17%) of cases were found to have loss of 9p, most commonly involving the whole arm. This loss showed a non-significant trend towards increased risk of relapse (p<0.1) in FH tumours taken at immediate nephrectomy, although there was no association with tumour stage. Loss of 9p was also observed in 4/19 (21%) of anaplastic tumours. Loss of copy number at the p16INK4a locus was confirmed in a small number of individual cases by the use of fluorescent in situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA). The loss of 9p in relapsing Wilms tumours was further investigated by loss of heterozygosity (LOH) analysis, with LOH detected at marker D9S1748 (p16INK4a) in 8/39 (20%) of tumours. Additional loci on 9p are currently being analysed for incidence of LOH. p16 protein expression was assessed on a Wilms tumour tissue microarray by immunohistochemistry. Overall, 12/82 (15%) tumours showed some degree of positivity staining for p16, with normal kidney uniformly negative. No significant difference in immunoreactivity was observed between different cell types, with blastemal, epithelial and stromal components showing similar patterns of immunoreactivity. There was no association between p16 protein expression and tumour stage or risk of relapse. Further studies are warranted to determine the significance of 9p loss in Wilms tumour.