Ahluwalia, A. [4]

Methylation: a predictor of chemotherapy response in ovarian cancer?

A. Ahluwalia[1], P. Yan[2], T.H.-M. Huang[2] & K.P. Nephew[1]

[1]*Medical Sciences, Indiana University School of Medicine, Bloomington, Indiana 47405, USA*
[2]*Department of Pathology, Ellis Fischel Cancer Center, University of Missouri–Columbia, Missouri 65203, USA*

Little is known about the molecular determinants of ovarian cancer, the fifth most common cause of cancer death in women. CpG island (CGI) hypermethylation is known to turn off gene expression and is associated with cancer progression. Our group used differential methylation hybridization, a microarray-based technique, to scan for alterations in methylation in ovarian cancer. We performed such screening of stage IIIC epithelial ovarian carcinomas (N=10), normal ovarian surface epithelial cells and ovarian cancer cell lines. A wide range of hypermethylated CGIs (2.5-fold increase; P <0.05) was seen in the ovarian carcinomas (23–98); however, the number of hypermethylated CGIs was similar across ovarian cancer cell lines (38–51). There were 12 hypermethylated CGIs (P<0.05) common to the ovarian cancer cell lines and tumors. Sequencing analysis revealed 11 methylated CGI tags in the ovarian tumors that had previously been shown to be hypermethylated in breast cancer. Tumor methylation levels before therapy were associated with patient response to chemotherapy (P=0.048; one-sided exact Wilcoxon rank sum test). Patients displaying a complete response had tumors with a low methylation score; conversely, patients with progressive disease had tumors with a higher methylation score. Although we have analyzed only a small fraction (2%) of the CpG islands in the human genome, we have found a relationship between the amount of methylation in ovarian tumors and response to chemotherapy. Our proof-of-concept study lays the foundation for genome-wide screening of methylation using DMH to examine epigenotype-phenotype relationships in ovarian cancer.

Ali, Shujath [5]

Genomic approaches to the development of prostate cancer diagnostics

Shujath Ali, Yongming Sun, Carrie Drumright, Luis Carretero, Christina Tran, Anton Nguyen, Dan Medynski, Yan Liu, Vu Viet Hoang, Brandon Liang, Herve Recipon, Robert Cafferkey & Roberto Macina

diaDexus, Inc., 3303 Octavius Drive, Santa Clara, California 95054, USA

Using bioinformatic and genomic approaches, we have identified several candidate genes that are differentially expressed in prostate cancer. The LifeSeq (Incyte Pharmaceuticals) DNA expressed sequence tag database consists of more than 6.4 million tags obtained from 1,317 complementary DNA libraries made from various tissues (normal and diseased). Using a bioinformatic approach, we looked for candidate genes that show a strong specificity for expression in prostate tissue and also a significant upregulation in cancerous tissue compared with normal tissue. In parallel we constructed several subtraction cDNA libraries using suppressive hybridization protocols (Clontech). These libraries are being sequenced and analyzed. To date a total of 81 genes have been chosen as candidates for further expression analysis. Expression analysis of these candidate genes involved the use of a sensitive Taqman quantitative polymerase chain reaction assay that further determined the tissue-specific expression and whether higher levels of expression are indicative of prostate cancer. Expression analysis data using RNA from different tissues and disease states (approximately 200 samples) helped rank these candidates. A total of eight candidate genes (*Arg2, Pro101, Pro108, Pro111, Pro118, Pro119, Pro121, Pro130*) were selected for further analysis. A majority of these have been cloned and expressed in bacteria. Monoclonal antibodies are available for two of them, and immunoassays will be developed shortly. These candidates, either individually or grouped in panels, have the potential to become new prostate cancer biomarkers for early detection, differential diagnosis, disease monitoring and disease surveillance.

Allander, Susanne [6]

Microarray-based genetic analysis of synovial sarcomas

Susanne Allander[1], Yidong Chen[1], Marc Ladanyi[2] & Paul Meltzer[1]

[1]*Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland, USA*
[2]*Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA*

Soft-tissue sarcomas are tumors of mesenchymal origin that often constitute a diagnostic and therapeutic dilemma. Some soft-tissue sarcomas are characterized by specific chromosomal translocations. The resulting fusion transcripts typically encode aberrant transcription factors, and their pathogenic effects are likely to be mediated through their actions on gene expression. Based on this hypothesis we are investigating gene expression patterns in synovial sarcoma using complementary DNA microarrays containing 6,500 sequence-verified human cDNAs. The t(X; 18)(p11.2; q11.2) translocation is characteristic of synovial sarcomas and fuses the *SYT* gene to either *SSX1* or *SSX2*. Expression analyses were performed on 16 synovial sarcomas confirmed to have either the *SYT–SSX1* or *SYT–SSX2* fusion transcript. Five other sarcoma samples, mostly malignant fibrous histiocytomas, were included as a comparison group. Hierarchical clustering analysis shows that these two tumor groups clearly separate with distinct expression patterns. One of the tumors, previously diagnosed as a synovial sarcoma, was closer to the malignant fibrous histiocytoma cluster. Analysis by means of the polymerase chain reaction with reverse transcription showed that this tumor was lacking the *SYT–SSX* fusion transcript, and the histological review reclassified it as a fibrosarcoma. This indicates that *SYT–SSX*-verified synovial sarcomas indeed have a specific expression profile. We are now attempting to determine the genes best able to define the synovial sarcoma cluster. Synovial sarcomas have two major histological subtypes, biphasic and monophasic, defined by the presence or absence of glandular epithelial differentiation in a background of spindle cells. Analyses of the genes discriminating monophasic from biphasic synovial sarcomas may also provide clues to the pathways regulating epithelial differentiation.

Alsner, Jan [7]

Detection of TP53 mutations by denaturing high-performance liquid chromatography

Jan Alsner, Susanne Berg Sorensen, Jesper Grau Eriksen & Jens Overgaard

Department of Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark

Recent insight into the clinical behavior of tumors harboring tumor protein p53 (*TP53*) mutations indicates that information concerning *TP53* can be useful in diagnosis, prognosis and choice of therapy for certain cancers. To detect mutations in clinical material, highly specific and sensitive mutation detection methods are