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whereas somatic inactivation of hMSH2 has rarely been found. In contrast, little is known about the overall involvement of hMSH6 in colorectal cancer. We investigated a series of 212 colorectal cancer specimens, comprising 141 sporadic cases and 71 cases fulfilling Bethesda guidelines for HNPCC for microsatellite instability, for protein expression of the four mismatch repair genes *hMSH6, hMSH2, hMLH1* and *hPMS2* by immunohistochemistry and for mutations by sequencing. We found different frequencies of abnormal gene expression for each mismatch repair protein studied. Among cases not fulfilling Bethesda guidelines, we identified hMLH1- and hMSH6-deficient cases. Sequence analysis identified hMSH6 germline mutations for almost all hMSH6-deficient cases. Lost expression of one or two of the four proteins was always associated with MSI-H tumors. Conversely, all except one of the MSI-H cases demonstrated lost or aberrant expression of one or more of the proteins, leaving little room for additional genes associated with the MSI-H phenotype. The combination of analysis of microsatellite instability and expression of the four mismatch repair proteins was highly predictive for the respective genes involved.

Plass, Christoph [7]

Contribution of DNA methylation to oncogenesis: Results of a genome scanning approach in multiple human tumors

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Aberrant promoter methylation is an epigenetic loss-of-function mutation analogous to point mutations or deletions. To study global methylation changes in human cancers, we use restriction landmark genomic scanning, a two-dimensional gel electrophoresis technique that allows the assessment of methylation patterns in up to 2,000 CpG islands per gel with methylation-sensitive restriction enzymes (NotI or AscI). Aberrant methylation in primary tumors is identified by comparing tumor profiles to profiles from matching normal DNAs. Loci aberrantly methylated in tumors are easily cloned using arrayed boundary libraries, and subsequent database searches allow us to link the methylation events to genes or expressed sequence tags. Important findings from our studies on cancers of human lung, head and neck; medulloblastomas; and acute myeloid leukemia include the following: (1) Identification of nonrandom, tumor-type-specific methylation events[1]. (2) Significant overrepresentation of methylated loci on chromosome 11 in acute myeloid leukemia. (3) Identification of six new target genes in lung cancer and ten in acute myeloid leukemia. (4) An increased number of methylation events in metastatic head and neck cancers with overlapping and new targets compared with primary tumors. (5) Methylation in the major breakpoint cluster region for medulloblastomas, suggesting a potential link between genetic instability and DNA methylation. Together these data suggest that the extent of DNA hypermethylation in cancer was previously underestimated and that epigenetic events have an outstanding potential for the identification of new tumor suppressor genes as well as diagnostic, prognostic and therapeutic targets.

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Pollock, Pamela M. [8]

Mutation analysis of the *CDKN2A* promoter in Australian melanoma families

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Approximately 50% of all melanoma families worldwide show linkage to 9p21–22; however, only about half of these families have been shown to contain a germline *CDKN2A* mutation. It has been proposed that a proportion of these families will carry mutations in the noncoding regions of *CDKN2A*. Several Canadian families were recently reported to carry a mutation, at position –34 relative to the start site, which gives rise to a new AUG translation initiation codon that markedly decreases translation from the wild-type AUG[1]. Haplotype sharing in these Canadian families suggested that this mutation might be of British origin. We have sequenced 0.4–1 Kb of the *CDKN2A* 5′ UTR and promoter in more than 300 Australian individuals with a family history of melanoma. Several known polymorphisms at positions –33, –191, –347, –493 and –735 were detected in addition to two new polymorphisms at positions –252 and –981 relative to the start codon. No individual was found to carry the previously characterized mutation at position –34 or any other disease-associated mutation. To investigate further noncoding *CDKN2A* mutations that affect transcription, allele-specific expression analysis was carried out in 33 families demonstrating either complete or indeterminate 9p haplotype sharing, in which one individual was heterozygous for at least one *CDKN2A* polymorphism. Polymerase chain reaction with reverse transcription and automated sequencing revealed expression of both *CDKN2A* alleles in all individuals tested. The lack of *CDKN2A* promoter mutations and absence of transcriptional silencing in the germ line suggest that noncoding *CDKN2A* mutations play a small role in melanoma predisposition.

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Porkka, Kati [9]

Detection of a new, prostate-specific gene by using suppression subtractive hybridization and cDNA library arrays

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Differential expression can be used to identify genes that are likely to be involved in the development and progression of cancer. In order to detect genes whose expression is decreased in prostate cancer, we combined two methods: suppression subtractive hybridization and complementary DNA library arrays. Screening of the subtracted cDNA library using array hybridization resulted in eight different clones that we confirmed to be truly differentially expressed. Seven of them represented known genes, and one of them was an anonymous expressed sequence tag (clone 1B10), matching the chromosomal region 7q21. In northern blot analysis, the expressed sequence tag 1B10 hybridized to a 7.5-kilobase transcript. The expression of 1B10 seems to be quite prostate-specific: by northern blot analysis it is expressed, in addition to prostate tissue, only in ovary tissue. By quantitative polymerase chain reaction with reverse transcription, the expression of 1B10 is also detected in other

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tissues, but at tenfold lower levels than in prostate. The only prostate cancer cell line that expresses 1B10 is an androgen-sensitive cell line, LNCaP, suggesting that the gene might be androgen-regulated. Cloning of the full-length cDNA has so far resulted in 4.6 kb of the transcript. The 3′ end of the coding region is significantly homologous to a prostate-specific transmembrane protein, STEAP, but the rest of the sequence does not show homology to any known gene. Cloning of the 5′ end of the cDNA and functional studies are now in progress.

Porter, Peggy L. [10]

Allelic imbalance in routinely processed breast tumors determined by affymetrix HuSNP array

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Analysis of allelic loss in archival tumor specimens using array technology is constrained by the quality and quantity of available tissue. A prototype Affymetrix HuSNP array was shown to provide reliable and reproducible assessment of allelic imbalance for 440 SNPs in frozen esophageal tumors (Mei-2000). However, the commercially-available Affymetrix HuSNP array (1,494 SNPs) has not been validated for the assessment of allelic imbalance in tumors processed by standard pathology methods. We tested the HuSNP assay in duplicate on breast specimens using both formalin-fixed and frozen, tumor and normal tissue taken from a single patient (16 arrays). Tumor cells were purified using bivariate cytokeratin/DNA flow sorting; normal breast served as constitutive normal. STR typing on 3 chromosomes validated regions of allelic imbalance. Allele calls from the HuSNP array averaged 95% reproducibility between duplicates and 94% concordance between the fixed and frozen samples. We also tested DNA from the same samples that was subjected to whole genome amplification (Primer Extension Pre-amplification, PEP) prior to array analysis. Although overall signal intensities were lower, the data from the PEPed material was reproducible in duplicates and concordant between sample types at similar rates to genomic DNA. Results from genomic normal tissue DNA averaged informative (AB) calls at 379 loci over all chromosomes. Although data points were clustered and large segments of chromosomes were not informative by this technique, our data indicated that the Affymetrix HuSNP assay could potentially provide a low resolution genome-wide analysis of allelic imbalance in routinely processed pathology specimens.

Randazzo, Filippo [11]

Cancer pathway analysis, DNA microarrays, and the identification of therapeutic targets

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We are discovering new drug targets for cancer using a combination of genomic and functional assay technologies. As part of our discovery and validation process we use DNA microarrays to determine the patterns of gene expression in normal tissue, primary tumors and metastatic tumors across a cancer patient population. Tens of thousands of genes, including cancer genes discovered by highly sensitive gene profiling technology, are placed on our DNA microchips. Our DNA microarray technology is highly sensitive and reproducible, allowing us to assay laser capture microdissected material from human biopsy samples. We analyze differential gene expression patterns in the cancer patient population using new clustering approaches. These patterns are, in turn, analyzed against our database of gene expression profiles obtained from cell lines in which pathways have been manipulated, and pathways that are altered in patients are identified. We then further evaluate selected differentially expressed genes, along with their pathways, as potential targets for diagnostic and therapeutic approaches using functional analysis.

Ratner, Nancy [12]

Gene expression analysis of NF1 mutant Schwann cells

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Schwann cells constitute the major cell population in neurofibromas, tumors that disfigure patients with the common inherited disease neurofibromatosis type 1. It is not known how abnormalities in neurofibroma Schwann cells contribute to tumor formation. We developed phenotypic assays that distinguish normal Schwann cells from Schwann cells (mouse and human) that lack the NF1 tumor suppressor protein, a GTPase-activating protein for Ras proteins. To define genes that may underlie these phenotypic changes we hybridized cDNA from wild-type and *Nf1* mutant mouse Schwann cells to microarrays from Incyte Genomics and compared gene expression levels. We carried out four arrays, each in duplicate, comparing cells of various phenotypes ($Nf1^{+/-}$, $Nf^{-/-}$, $Nf1^{-/-}$TXF [a proliferative Schwann cell population] and $Nf1^{-/-}$TXF treated with a farnesyl transferase inhibitor [a drug class in clinical trials in NF1] to wild-type Schwann cells. Thirteen changes of greater than fourfold were identified in $Nf1^{-/-}$ Schwann cells. Larger numbers of genes were differentially expressed in $Nf1^{-/-}$TXF Schwann cells; some were sensitive to farnesyl transferase inhibitor. We report on the reproducibility of the data and confirm the RNA and protein levels for some identified genes. We have also begun an effort to share array data among laboratories studying neurofibromatosis as part of an international consortium. By applying these methods to NF1 model systems we hope to learn how neurofibromin serves as a tumor suppressor and to identify potential biomarkers and therapeutic targets for improved management of neurofibromatosis.

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