

**Abstracts: Session III**

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.

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Contribution of DNA methylation to oncogenesis: Results of a genome scanning approach in multiple human tumorsChristoph Plass¹, Michael C. Frühwald^{1,2}, Laura J. Rush^{1,3}, Zunyan Dai¹, Laura T. Smith¹ & Dominic J. Smiraglia¹¹Division of Human Cancer Genetics, Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, Columbus, Ohio, USA²Pädiatrische Hämatologie/Onkologie, Klinik und Poliklinik für Kinderheilkunde, Westfälische Wilhelms Universität Münster, Münster, Germany³Department of Veterinary Biosciences, Ohio State University, Columbus, Ohio, USA

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 events¹. (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.

1. Costello, J.F. *et al. Nature Genet.* **25**, 132–138 (2000).

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[8]

Mutation analysis of the *CDKN2A* promoter in Australian melanoma familiesPamela M. Pollock¹, Mitchell Stark², Jane M. Palmer², Marilyn K. Walters², Nick G. Martin², Adele C. Green² & Nicholas K. Hayward²¹Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892, USA²Queensland Institute of Medical Research, P.O. Royal Brisbane Hospital, Queensland 4029, Australia

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 AUG¹. 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.

1. Liu, L. *et al. Nature Genet.* **21**, 128–132 (1999).

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