stitutively operational in cultured cells. However, p53 does not regulate expression of *Mdm2* constitutively in adult murine tissues. Instead p53 requires a stimulus, such as whole-body ionizing irradiation, to stimulate *Mdm2* expression. These results indicate that p53 regulates *Mdm2* expression only under certain conditions of stress. We propose that MDM2 regulates p53 only under conditions in which p53 regulates *Mdm2*. To test our hypothesis, we have generated mice carrying a conditionally null allele of *Mdm2*. We have excised exons 7–9 in the germ line and shown that the conditional allele functions as a wild-type allele before excision and as a null allele after excision. We will report whether loss of *Mdm2* in adult tissues increases p53 levels and activities constitutively or only following a stressor, such as whole-body ionizing irradiation. This study has clinical relevance since the interaction of p53 and MDM2 has become a target for new anticancer agents.

Peters, Mette A. [4]

Genome-wide scan for high-risk prostate cancer families with breast cancer reveals new loci for prostate cancer and breast cancer

Mette A. Peters[1], Janet L. Stanford[1], Mike D. Badzioch[2], Suzanne Kolb[1], Marta Janer[3], Ellen L. Goode[2], Mark Gibbs[1], Leroy Hood[3], Elaine A Ostrander[1] & Gail P. Jarvik[2]

[1]*Fred Hutchinson Cancer Research Center, Seattle, Washington, USA*
[2]*University of Washington, Seattle, Washington, USA*
[3]*Institute for Systems Biology, Seattle, Washington, USA*

Hereditary prostate cancer is a complex disease, and in some families it seems to segregate with other cancers. Epidemiological and genetic studies have reported familial clustering of prostate and breast cancers. As part of a genome-wide search for prostate cancer genes, we performed linkage analyses in 27 families with three or more affected men with prostate cancer who had at least one first-degree relative with breast cancer. To maximize homogeneity, we stratified these families by the pattern of breast cancer (one case, n=14; two cases, n=6; any ovarian cancer, n=7). Families were stratified into early- (66 yr) or later-onset (= 66 yr) prostate cancer on the basis of median age at diagnosis. GENEHUNTER was used to compute multipoint NPL scores for prostate cancer linkage using 380 genomic scan markers. Stratified analyses revealed three chromosomal regions with NPL scores of 3.0: (1) chromosome 7q, NPL=3.98 (P=0.002) at markers D7S1826–D7S1805 in later-onset prostate cancer families with breast and ovarian cancer; (2) chromosome 14q, NPL=3.47 (P=0.01) at marker D14S587 in early-onset prostate cancer families with more than two first-degree relatives with breast cancer and (3) chromosome Xq, NPL=3.12 (P=0.003) at marker GATA172D05 in later-onset prostate cancer families with only one relative with breast cancer. None of these families had evidence of significance linkage on chromosomes 13 or 17. These data supply further evidence for prostate cancer susceptibility genes on chromosomes 14 and X, and they highlight a new region of interest on chromosome 7 that may be involved in the etiology of both prostate cancer and breast cancer.

Petros, John [5]

Classification of adult renal epithelial neoplasms by gene expression profiling using cDNA microarrays

Petros, John[1,2,3], Andrew Young[1], Mahul Amin[1], So-Dug Lim[1], Fray Marshall[1,2], James Madara[1] & Andrew Neish[1]

[1]*Departments of Urology and Pathology, Emory University, Atlanta, Georgia, USA*
[2]*Winship Cancer Institute, Atlanta, Georgia, USA*
[3]*Atlanta Veterans Administration Medical Center, Atlanta, Georgia, USA*

We analyzed expression of 7,075 genes in four conventional renal cell carcinomas (RCCs), one chromophobe RCC, and two oncocytomas using complementary DNA microarrays. Expression profiles were compared between tumors using hierarchical clustering algorithms. Tumors segregated into two major gene expression classes correlated with histopathological diagnoses; specifically, conventional RCCs were clearly distinguished from chromophobe RCCs and oncocytomas. We classified tumors with high resolution using a selected panel of 70 differentially expressed genes. Chromophobe carcinoma and oncocytomas overexpressed strikingly similar genes, including several related to oxidative phosphorylation and several expressed normally by the distal nephron; these findings are consistent with the mitochondrion-rich morphology of these tumors and the theory that both lesions are related histogenetically to distal nephron epithelium. Conventional RCCs underexpressed mitochondrial and distal nephron genes, and they were further distinguished from chromophobe RCCs and oncocytomas by overexpression of vimentin and molecules related to class II of the major histocompatibility complex. Expression patterns of four genes (those coding for vimentin, CD74, parvalbumin, and galectin-3) were validated in 34 additional tumors by immunohistochemistry. Vimentin was a sensitive, specific marker for conventional RCCs, and parvalbumin was a promising marker for chromophobe RCCs and oncocytomas. Gene expression profiling identified distinct expression patterns related to the pathobiology of renal neoplasms and was an effective approach for discovering immunomarkers for renal tumor subtypes.

Plaschke, Jens [6]

Near-complete association between microsatellite instability and abnormal immunostaining of the mismatch repair proteins hMSH2, hMSH6, hMLH1 and hPMS2 and involvement of hMSH6 in sporadic and hereditary colorectal cancers

Jens Plaschke[1], Stefan Krüger[1], Stephan Haas[1], Steffen Pistorius[2], Hans-D. Saeger[2] & Hans K. Schackert[1]

[1]*Department of Surgical Research, Technical University of Dresden, Dresden, Germany*
[2]*Department of Visceral, Thoracic and Vascular Surgery, Technical University of Dresden, Dresden, Germany*

Germline mutations in human mismatch repair genes (mostly in hMSH2 and hMLH1, and recently also in hMSH6) have been found to be associated with the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome. HNPCC tumors are phenotypically characterized by a high level of microsatellite instability (MSI-H). Moreover, about 10–15% of sporadic colorectal cancers are MSI-H, predominantly on the basis of epigenetic silencing of hMLH1 by promoter methylation,

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

Christoph Plass[1], Michael C. Frühwald[1,2], Laura J. Rush[1,3], Zunyan Dai[1], Laura T. Smith[1] & Dominic J. Smiraglia[1]

[1]*Division of Human Cancer Genetics, Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, Columbus, Ohio, USA*
[2] *Pädiatrische Hämatologie/Onkologie, Klinik und Poliklinik für Kinderheilkunde, Westfälische Wilhelms Universität Münster, Münster, Germany*
[3]*Department 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 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.

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

Pollock, Pamela M. [8]

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

Pamela. M. Pollock[1], Mitchell Stark[2], Jane M. Palmer[2], Marilyn K. Walters[2], Nick G. Martin[2], Adele C. Green[2] & Nicholas K. Hayward[2]

[1]*Cancer Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892, USA*
[2]*Queensland 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 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.

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

Kati Porkka & Tapio Visakorpi

Laboratory of Cancer Genetics, Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland

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