Abstracts: Session III

downward approach; it has yielded only targets at low levels within the replication machinery, never at the level of proliferation control and signaling proteins. Drug-like molecules with molecular weights around 500 seem to work well as inhibitors of tyrosine kinases, docking in their ATP pocket; however, molecules of this kind are perhaps too compact for selective interference at the level of cross-talks between the domains of two signaling proteins. Rapid evolution has recently taken place in possibilities for internalizing amphipathic molecules of molecular weights of several thousand. Under these conditions it becomes possible to conceive peptidomimetic drugs of several dozen amino-acid-like units, and these are much better suited for such selective interference. Small, dedicated peptidomimetic combinatorial libraries, based on a natural protein motif involved in the cross-talk of interest and on computer modeling, are natural allies in this game. A recently developed peptidomimetic lead capable of interfering with Myc activity^{1,2} will be discussed within the framework of this new strategy for developing antineoplastic drugs.

¹ Pescarolo, M.P. *et al.* *Cancer Res.* **58**, 3654–3659 (1998).
² Pescarolo, M.P. *et al.* *FASEB J. Exptl.* January 2001.

Paules, Richard S.

[1]

ATM-dependent responses to DNA-damaging agents

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Ataxia telangiectasia is an autosomal recessive disorder characterized by neuronal degeneration, telangiectasias, acute cancer predisposition and hypersensitivity to ionizing radiation (IR). The gene defective in this disorder, *ATM* (for AT-mutated), encodes a protein, pATM, that has been found to have IR-inducible kinase activity. Cells from individuals with AT exhibit severely attenuated cell cycle checkpoint function in response to IR exposure and are hypersensitive to IR-induced killing. It has been suggested that pATM acts as part of a complex that senses DNA damage and in particular DNA double-strand breaks; it has also been speculated that pATM participates in response to oxidative damage. We have been studying pATM-dependent cellular responses to various DNA-damaging agents. As part of this effort, we are investigating global gene expression changes following exposures to IR in both lymphoblast and fibroblast cells from multiple individuals with either normal or defective pATM function. We are comparing gene expression changes in both normal and pATM-deficient cells from one cell type that is predisposed to undergo apoptosis (lymphoblasts) with those in cells predisposed to undergo a prolonged cell cycle arrest (fibroblasts) following IR exposure. In addition, we are comparing these responses to IR in normal and pATM-deficient fibroblasts with responses to exposure to ultraviolet light in the 000- to 000-nm range and reactive oxygen species. These analyses are being performed using National Institute of Environmental Health Sciences Human ToxCips, with approximately 2,000 known complementary DNA clones, as well as with Human Discovery Chips, a collection of approximately 12,000 known and anonymous cDNA

Pedersen, Tanja X.

[2]

Profiling changes in keratinocyte gene expression during wound reepithelialization by laser capture microdissection combined with cDNA array analysis

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Incisional wounding of the skin triggers a series of dramatic events in the epidermal keratinocytes that are located immediately adjacent to the margins of the wound, ultimately leading to complete reepithelialization of the wound by these cells. These events include hyperproliferation of basal keratinocytes, dissolution of cell–cell adhesions, detachment from the basement membrane, lateral migration into the wounded area, invasion and proteolytic degradation of the provisional matrix of the wound bed. In many aspects, the healing response resembles the phenotypic events observed during squamous carcinoma progression, in which normal keratinocytes undergo a malignant conversion to acquire a proliferative, migratory and invasive phenotype. To elucidate the molecular mechanisms underlying the transformation of keratinocytes to a migratory and proteolytic phenotype, we initiated a study of global changes in keratinocyte gene expression during mouse incisional skin wound healing. We used laser capture microdissection, which allows the procurement of pure cell populations from heterogeneous histological samples, to isolate wound keratinocytes that actively migrate through and degrade the provisional matrix of full-thickness incisional mouse skin wounds. For comparison, we isolated nonmigrating keratinocytes distal to the wound edge, as well as keratinocytes from mock-wounded mice, in a similar manner. We isolated total RNA (11–18 ng) from approximately 5,000 keratinocytes and generated complex complementary DNA probes by reverse transcription of the messenger RNA fraction. We then screened cDNA expression arrays to identify the expression pattern of 1,176 mouse genes in the three populations of keratinocytes. Results of the expression studies will be presented and discussed.

Perry, Mary Ellen

[3]

Conditional inactivation of *Mdm2*

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The *mdm2* oncogene encodes p90MDM2, a critical negative regulator of the p53 tumor suppressor protein. The early embryonic lethality of mice of *Mdm2* null genotype precludes an evaluation of MDM2's role in regulating p53 in adult tissues. It is critical to understand the mechanisms regulating levels and activities of p53, because loss of p53 function leads to tumorigenesis whereas high levels of active p53 can stimulate apoptosis. The ability of p53 to regulate the expression of its own inhibitor, p90MDM2, has led to the suggestion that p53 controls its own activity through a negative feedback loop with *Mdm2*. This feedback loop is con-

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

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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

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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

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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,