INTERMEDIATE BIOMARKERS OF PRECANCER AND THEIR APPLICATIONS IN CHEMOPREVENTION

Organizers: Martin Lipkin, Bernard Levin, Young S. Kim and Gary J. Kelloff

October 3-7, 1991

Plenary Sessions Page

Poster Sessions

Intermediate Biomarkers of Precancer and their Application in Chemoprevention

Keynote Address

WA 001 COLONIC BIOMARKERS, Martin Lipkin, M.D., Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Modified cell proliferation, differentiation and gene structure and expression have been identified in colonic epithelial cells in diseases leading to increased frequencies of neoplasia. Mutations, altered gene expression, expansions in proliferative compartment size, deletions of portions of the genome and many other characteristics of abnormal cell differentiation and maturation occur.

Proliferating cells can now be identified in biopsy sections (a) by incorporating tritiated thymidine ([^{3}H]dThd) or (b) BrdU into newly synthesized DNA of S phase cells; by identifying an increasing number of antigens including (c) proliferating cell nuclear antigen (PCNA) in cells in S and other cell cycle phases, and (d) Ki67 in multiple cell cycle phases.

In a recent study in normal colon BrdU and PCNA were comparable to [^{3}H]dThd as reliable markers of proliferating epithelial cells[1]. A recent study combining multiple biomarkers[2] has shown an expanded proliferative compartment together with abnormal expression of cytoskeletal-associated proteins which may indicate a more advanced precancerous stage of ulcerative colitis. Using BrdU in human subjects to measure cell proliferation[3], labeling indices in colonic crypts were higher in subjects with previous adenomas or colon cancer than in subjects without lesions; the major zone of DNA synthesis shifted towards the crypt surface. A panel of 30 cDNA clones has been identified[4] whose pattern of expression in individual biopsies distinguished flat, normal appearing colonic mucosa of patients in two high-risk groups from low-risk subjects. The two high risk groups, familial polyposis and hereditary non-polyposis colon cancer had similar patterns of expression of the 30 selected clones.

These various intermediate biomarkers are now being applied to chemoprevention studies, increasing the ability of investigators to analyze the effects of novel chemopreventive agents in the colon and other organs.

[1]Richter F et al, Proc Amer Assn Canc Res 32:26, 1991; [2]Paganelli GM et al, Gastroenterol 98: A302, 1990; [3]Risio M et al, Cancer Res 51:1917, 1991; [4]Augenlicht L et al, Proc Natl Acad Sci USA 88:3286, 1991.

Introductory Session

WA 002 THE NATURAL HISTORY OF INTRAEPITHELIAL NEOPLASIA -IMPLICATIONS FOR BIOMARKER APPLICATION, Charles W. Boone, Gary J. Kelloff, and Vernon E. Steele, Chemoprevention Branch, Division of Cancer Prevention and Control, National Cancer Institute, NIH, Bethesda, MD 20892

Epithelial neoplasia typically has a preinvasive intraepithelial stage that lasts for years. A better understanding of human intraepithelial neoplasia is of critical importance to the chemoprevention field not only because it is the target condition for which drugs must be sought to slow or stop the process, but also because the cell and tissue abnormalities characteristic of intra-epithelial neoplasia form the basis for the development of many types of intermediate endpoint biomarkers. As defined by many authors, mild, moderate, and severe dysplasia, considered biologically equivalent to intra-epithelial neoplasia, appear to be basically the same in all squamous and columnar human epithelia. Aneuploidy frequently occurs during intraepithelial neoplasia in humans and establishes the phenotypic heterogeneity required for clonal evolution to occur during this preinvasive stage. From a survey of the literature there appear to be 7 basic morphological criteria of intra-epithelial neoplasia. Each criterion is itself a biomarker and may be related to other established biomarkers, as follows:1) Increased nuclear size (measured by flow or image cytometry), 2) Abnormal nuclear shape (measured by cytomorphometry), 3) Increased nuclear stain uptake (Feulgen nuclear stain uptake measured by cytospectro-photometry detects aneuploidy), 4) Nuclear pleomorphism (estimated by increased variance of the mean nuclear area), 5) Increased mitoses (related to proliferation markers), 6) Abnormal mitoses (a marker of aneuploidy), 7) Disordered or absent epithelial maturation (related to differentiation markers). From the above it is clear that an intermediate endpoint biomarker may be defined as a measureable cellular or molecular property associated with, or occurring prior to, intraepithelial neoplasia.

WA 003 STATISTICAL CONSIDERATIONS IN EVALUATING INTERMEDIATE ENDPOINTS,

Laurence S. Freedman, Arthur Schatzkin, Mark H. Schiffman, National Cancer Institute, Bethesda, MD 20892.

Discovering biological markers that are true intermediate endpoints, i.e. lie on major causal pathways to cancer, is a high priority in cancer research. Such intermediate endpoints would provide new insights into etiology, new direction to prevention and would facilitate the design of cancer prevention studies. We present strategies for determining whether a biological marker is a valid intermediate endpoint between an exposure or intervention and cancer incidence. Two important statistical concepts underlying the validation are the attributable proportion and the exposure or intervention effect adjusted for the biological marker. Validation may be carried out by including the biological marker measurement within the context of an intervention or observational study where cancer incidence is the endpoint. Examples of HPV infection in cervical cancer and serum cholesterol in coronary heart disease are given to illustrate these concepts.

WA 004 CURRENT CLINICAL TRIALS IN CHEMOPREVENTION WITH BIOMARKERS, Waun K. Hong, Scott M. Lippman, Jin S. Lee, and Walter N. Hittelman, Department of Medical Oncology, U.T. M.D. Anderson Cancer Center, Houston, TX 77030

The concepts of field carcinogenesis and multistep tumorigenesis support the study of chemoprevention within the aerodigestive tract. Strong preclinical and clinical data support the study of retinoids for epithelial chemoprevention. Our current chemoprevention trials include randomized studies in oral premalignancy (low-dose 13-cis-Retinoic Acid [13cRA] versus beta-carotene maintenance) and bronchial metaplasia (13cRA vs Placebo). A major new focus of these trials is the integration of intermediate endpoint biomarker (IEBM) studies. These markers are short-term estimates of tissue specific biologic activity of potential chemopreventive agents which can greatly enhance chemoprevention study design by identifying high risk sites and providing strong rationale for full scale phase III trials. Our group is studying a spectrum of genetic markers, both non-specific markers of DNA damages (micronuclei) and specific chromosomal alterations. Using in situ (chromosome painting) techniques, we have been able to identify genetic alterations within premalignant lesions. Phenotypic studies of proliferation and differentiation have also yielded promising early results. We observed a significant correlation between altered proliferation patterns and histologic progression from normal to hyperplastic to metaplastic and dysplastic lesions within the bronchial epithelium. We also observed altered expression of blood group antigen A in premalignant lesions and preliminary data suggest modulation of the expression of this differentiation antigen by 13cRA. We are now expanding these marker studies in our new long term trials to include a series of growth regulatory genes (e.g., TGF-ß, EGFR. c-myc, P53). These studies will generate important data on the biology of aerodigestive tract carcinogenesis and identify promising candidate intermediate biomarkers.

WA 005 GASTROINTESTINAL CANCER: RISK FACTORS, PATHOGENESIS AND THE DEVELOPMENT OF BIOMARKERS FOR CHEMOPREVENTION STUDIES, Martin Lipkin, M.D., Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Dietary, environmental and genetic factors contribute to the etiology, pathogenesis and risk for gastrointestinal cancers. In the colon, dietary fat, fiber, calcium, other micronutrients, inflammatory diseases and familial cancer associations modify risk. In the stomach and esophagus, dietary irritants, nutritional deficiencies and bacterial growth and products modify pathogenesis and level of risk.

Measurements of cell proliferation and differentiation within the gastrointestinal tract have now identified abnormal cellular characteristics associated with increased susceptibility to gastrointestinal cancer. In precancerous diseases of the esophagus the proliferative compartment progressively increases in size, increased ploidy and dysplasia develop, and epithelial cells express abnormal cytokeratins and ectopic tumor-associated antigens. In the stomach increased proliferative activity and metaplasia and dysplasia develop, poorly differentiated cells line the gastric surface, intestinal enzymes and mucins are expressed and normal gastric antigens are replaced by intestinal or embryonic antigens.

In colonic adenomas a massive shift of the entire proliferative compartment to the polyp surface can occur. In flat colonic mucosa expansions of the size of the proliferative compartment also occur in diseases that lead to increased frequencies of human colorectal cancer, and DNA content increased in upper-third nuclei of colonic crypts. Gene expression is modified, deletions of portions of the genome occur, and blood-group-related antigens are modified as the cells undergo abnormal differentiation and develop into adenomas and carcinomas.

Chemopreventive regimens are now being tested to determine whether they modify intermediate biomarkers towards normalization characteristic of lower risk for neoplasia. It is anticipated that in future attempts to prevent the development of gastrointestinal cancer, the application of intermediate biomarkers to chemoprevention studies may permit more novel chemopreventive regimens to be tested in human subjects than heretofore was possible.

Colon Markers-I: Preneoplastic Lesions

WA 006 THE ADENOMA-ADENOCARCINOMA SEQUENCE IN THE LARGE BOWEL: VARIATIONS ON A THEME. Stanley R. Hamilton, Department of Pathology and Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Most adenocarcinomas of the colorectum arise in a visible benign precursor lesion, the adenoma, which is a monoclonal proliferation of dysplastic nonmalignant epithelial cells. The resultant adenoma-adenocarcinoma sequence represents the predominant pathogenetic pathway, in contrast to "de novo carcinoma". Therefore, the adenoma is a tempting endpoint for chemoprevention trials.

The adenoma-adenocarcinoma sequence occurs in diverse clinical settings. In familial adenomatous polyposis syndrome (FAP), autosomal dominant inheritance of the mutated APC (adenomatous polyposis coli) gene on chromosome 5q21 typically results in thousands of adenomas in the colorectum and lesser numbers in the proximal small bowel. Adenocarcinoma usually develops in only a few of the many adenomas, typically in the left colon and duodenum. In hereditary nonpolyposis colorectal cancer syndrome (HNPCC), autosomal dominant inheritance of an as yet unidentified gene appears to result in small numbers of adenomas which progress frequently to adenocarcinoma, predominantly in the right or transverse colon. In familial aggregation of colorectal cancer without a recognizable syndrome, cancer and/or adenomas occur in pedigree members. In "sporadic" cancers and adenomas family history is absent and the tumors are mainly in the left colon.

Colorectal adenomas have variable characteristics including size, shape (polypoid vs. flat), villous architecture, and dysplasia. A variety of oncogenes and tumor suppressor genes are altered during progression. Epigenetic factors are important as evidenced by disappearance of adenomas in FAP patients after ileorectal anastomosis or the nonsteroidal anti-inflammatory drug sulindac.

Several variations on the theme of the adenoma-carcinoma sequence are evident. Identification of the inherited and acquired genetic alterations as well as the interacting environmental factors will provide a rational basis for chemoprevention. Conversely, effective chemopreventative agents will contribute to understanding of the critical pathogenetic mechanisms.

WA 007 PROGRESSION TO CARCINOMA IN ULCERATIVE COLITIS: BIOLOGY AND CLINICAL IMPLICATIONS,

Bernard Levin, Department of Medical Oncology, The U.T. M.D. Anderson Cancer Center, Houston, Texas 77030.

The risk of colorectal carcinoma is increased among patients with longstanding ulcerative colitis and Crohn's disease. The development of cancer in inflammatory bowel disease is hypothesized to evolve by a multistep process involving genetic instability, clonal expansion and the development of a malignant phenotype. The contribution of nutritional factors such as folate deficiency[1] is of great interest; molecular genetic mechanisms are under study[2]. In contrast to sporadic colorectal carcinoma, carcinomas in ulcerative colitis are associated with a long prior history of chronic inflammation and the subsequent development of epithelial dysplasia. Dysplasia is defined as an unequivocal neoplastic alteration of the colonic mucosa[3]. The object of surveillance is prevention of death from cancer by detection at a pre-malignant or early curable stage. Patients at greatest risk of cancer who customarily undergo endoscopic surveillance are those with extensive colitis of more than 8 years duration. Dysplastic epithelium may occur in flat mucosa, may produce a plaque or a nodular/villiform appearance. Dysplasia is not present in all patients with cancer in colitis. It is important to develop more sensitive and specific markers for the presence of precancer or cancer in colitis. Under study are proliferation associated markers detected by immunohistochemistry[4], lectin binding[5], flow cytometry[6] and laser induced fluorescence coupled with endoscopy.

1. Rosenberg IH, Mason JB: Folate, dysplasia and cancer. Gastroenterology 97:502-505, 1989.
2. Burmer GC et al: c-Ki-ras mutations in chronic ulcerative colitis and sporadic colon carcinoma. Gastroenterology 99:416-420, 1990.
3. Riddell RH et al: Dysplasia in inflammatory bowel disease: standardized classification with provisional clinical applications. Hum Pathol 14:931-968, 1983.
4. Wargovich MJ: Specific laboratory markers of colonic epithelial cell proliferation. IN: Riddell RH (ed): Dysplasia and Cancer in Colitis. New York, 1991, p147-152.
5. Boland CR et al: Use of the lectin from Amaranthus caudatus as a histochemical probe of proliferating colonic epithelial cells. Cancer Res 51:657-665, 1991.
6. Ahnen DJ et al: Can dysplasia be defined objectively? IN: Riddell RH (ed): Dysplasia and Cancer in Colitis.New York, 1991, p255-262.

WA 008 ABERRANT CRYPTS IN HUMAN COLONIC MUCOSA: PUTATIVE PRENEOPLASTIC LESIONS, Theresa P. Pretlow, Mary Ann O'Riordan, Thomas G. Pretlow, and Thomas A. Stellato, Departments of Pathology and Surgery, Case Western Reserve University School of Medicine, Cleveland, OH 44120

Aberrant crypts were first defined by Bird (1) in methylene-blue stained, unsectioned, colonic mucosa from carcinogen-treated rodents by their increased size, eliptical luminal opening, thicker epithelial layer, and increased pericryptal region. In rodents, aberrant crypts are observed as early as 2 weeks and for at least 9 months after a single dose of carcinogen, are not observed after doses of toxic noncarcinogenic compounds, and have a distribution that parallels that of tumors. The number of aberrant crypts per focus does not increase with dose of carcinogen but does increase with time after the carcinogen dose. The number of foci of aberrant crypts is dose dependent and can be modified by compounds that alter the incidence of colon cancer. The ability to quantify these lesions in the entire colon in less than an hour suggests that aberrant crypts may be a highly efficient in vivo bioassay for colon carcinogens. Since aberrant crypts appear to be the earliest identifiable putative precursors of colon cancer, they provide lesions that can be further characterized for the earliest genetic and biochemical alterations. In F344 rats, we have demonstrated that aberrant crypts have multiple histochemically demonstrable enzyme alterations (2). Using similar techniques, we were the first to demonstrate aberrant crypts in unsectioned human mucosa (3). Microscopically (3,4) these aberrant crypts, after embedding and sectioning, resemble rare lesions described earlier in the literature after extensive serial sectioning. In rats and humans, aberrant crypts display varying degrees of dysplasia and histochemically demonstrable altered enzyme activities. These putative, preneoplastic lesions should provide helpful insights into early changes that precede colon cancer and ways to alter their progression.

1. Bird, R.P., Cancer Lett 37: 147-151, 1987.
2. Pretlow, T.P. et al., Am J Pathol 136: 13-16, 1990.
3. Pretlow, T. P. et al., Cancer Res., 51: 1564-1567, 1991.
4. Roncucci, L. et al., Hum. Pathol. 22: 287-294, 1991.

WA 009 **GROWTH KINETICS AND CHEMOPREVENTION OF ABERRANT CRYPTS IN THE RAT COLON**, Michael J. Wargovich, Charles Harris, Chi-Dai Chen, Cynthia Palmer, Vernon Steele, and Gary Kelloff. Department of Medical Oncology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030 and Chemoprevention Branch, National Cancer Institute, Bethesda, MD 20892.

Single to multiple colonic crypts exhibiting dysplasia and detectable in situ by staining of rat colon with methylene blue are called aberrant crypts (AC) and may serve as an intermediate marker for colon cancer. In a characterization study we have established the kinetics of AC growth and development over a period of 20 d following injection of rats with the carcinogen, azoxymethane (AOM). AC are not present at 5d post-injection, but are a constant feature at 10d and thereafter. Multiple AC, assumedly clonal, begin to evolve at 10d and are consistent by 20d, forming incipient microadenomata. We have examined 20 candidate chemopreventives for inhibition of AC. All agents were given in AIN-76 diet, at two dose levels, during the period AOM was injected. AC were measured after 5 weeks of growth. Among the most active agents in inhibiting AC were BHA, DFMO, quercetin, diallyl sulfide, 18ß glycyyrhetenic acid, and ascorbyl palmitate. In a post-initation study the differentiation agent, sodium butyrate was ineffective, but piroxicam was highly effective in modulating AC growth. Further prioxicam inhibited AC development at all stages of growth from single to polycryptal clusters of AC. The AC assay shows marked sensitivity and specificity for screening agents for chemoprevention of colon cancer. Supported by NCI NO1-CN-85101-01.

Colon Markers-II: Proliferation Markers

WA 010 ***BIOMARKERS OF CELL PROLIFERATION IN THE GASTROINTESTINAL TRACT.*** *Guido Biasco, Gian Maria Paganelli, Luigi Barbara. "Giorgio Prodi" Center for Cancer Research, University of Bologna, Bologna, Italy*

Several studies indicate that cell proliferation and differentiation abnormalities are related to an increased risk of cancer. In the mucosa of the gastrointestinal tract these abnormalities are expressed by two main phoenomena: an increase of the cell turnover rate and a defective maturation of epithelial cells. The two patterns do not always appear together. This could mean that they are due to different biological mechanisms. In some clinical conditions at high risk of gastrointestinal cancer (i.e., chronic atrophic gastritis, colorectal adenoma or cancer, chronic ulcerative colitis) we observe an expansion of the proliferative compartment even if the mucosa is not affected by morphological abnormalities (1, 2, 3). This proliferative feature seems to be associated to the presence of defects of cell differentiation (4). Moreover, the magnitude of the error, or, in other terms, the number of proliferating cells in mucosal areas normally containing only mature elements, seems to express the level of cancer risk (2). On the contrary, the increase of cell turnover rate is not constant. These observations suggest that the most sensitive and probably earliest cell defect is represented by an error of the control of both cell growth and maturation. The abnormality is well detected by the histological examination of the proliferative pattern. The most reliable methods are microautoradiography after incorporation of tritiated thymidine (^{3}H-Thd), immunohistochemistry after bromodeoxyuridine (BrdU) uptake, and probably the ultimate immunohistochemistry using anti-proliferating cell nuclear antigen (PCNA) monoclonal antibodies on routinely-fixed tissue. In particular, ^{3}H-Thd or BrdU incorporation may constitute the gold standard for the evaluation of other techniques. The literature, and our own results, indicate that the search for abnormalities of epithelial cell proliferation can be useful for: *a)* the study of the earliest mechanisms leading to gastrointestinal cancer, *b)* the detection of subjects at high cancer risk or the definition of the individual risk level, *c)* pilot chemoprevention studies using these abnormalities as intermediate biomarkers of gastrointestinal cancer risk.

1) Biasco G et al, Ital J Gastroenterol 1991; 23: 24-28
2) Paganelli GM et al, Cancer (in press)
3) Biasco G et al, Cancer Res 1990; 50: 1156-1159
4) Biasco G et al, Cancer Res 1984; 44: 5450-5454

WA 011 COLON BIOMARKERS, M. Risio - Dept. of Pathology - Ospedale S. Giovanni A.S. - Torino-Italy. Renewal of mucosa cells throughout the large intestine is normally governed by a strictly compartmentalized model, in which proliferation of undifferentiated cells is confined to the deepest parts of the crypts, while differentiation is accompanied by a continuous, progressive and orderly migration to the surface. Identification of S-phase cells by their uptake of 3H-thymidine or bromodeoxyuridine is the method of choice for detailed compartmental mapping of proliferation. Immunohistochemical detection of proliferation-associated antigens (Ki67, PCNA) is not sufficiently reliable, though capable of disclosing gross changes in proliferation as a whole. Division of each hemicrypt into five equal longitudinal compartments from the base (compartment 1) to the mouth (compartment 5) is a practical way of quantifying proliferation levels and distribution. The cytokinetic profile is provided by the total labeling index (TLI), the compartment labeling indices (LI 1-5), the compartment labeling percentages (P 1-5), and the mean compartment distribution of proliferating cells (C1-5). Inflammation leads to local hyperproliferation that may be transient (self-limited colitis, Crohn's disease, acute radiation damage) or lasting (ulcerative colitis). Proliferating cells are then uniformly distributed along the crypt. A marked alteration in proliferating cell distribution is a late feature of the irradiated rectal mucosa and in sub-groups of ulcerative colitis with a higher risk of cancer. It corresponds to the progressive shift of the major zone of DNA synthesis from the base to the intermediate and superficial compartments (stage II abnormality). Epithelial renewal is greatly altered in colorectal neoplasia and the cytokinetic derangement involves the entire intestinal mucosa, despite the focal nature of the preneoplastic or neoplastic lesion. Hyperproliferation is progressively exacerbated in non-familial neoplasia (adenoma < adenocarcinoma < adenoma+adenocarcinoma), whereas the stage II abnormality is more marked in hereditary colorectal neoplasia. Proliferation levels are correlated with size (TLI and LI 1-5 close to normal when an adenoma is < 1cm) and with the chronology of preneoplastic lesions (TLI and LI 1-5 decrease in function of persistence of the polyp-free colon state). These factors do not alter the location of the proliferative compartment, whose shift to the surface is linked to the histological features of pre-neoplastic tissue (dysplasia) or the adenoma+adenocarcinoma association. Compartmental differences in the level of expression of Epidermal Growth Factor receptors in S-phase cells allow the succession and interactions of these cytokinetic abnormalities to be predicted. These abnormalities are also correlated with the histogenetic sequences of colorectal carcinoma. They are, in fact, an early step in the genesis of colorectal adenoma and a highly predictive marker of the adenoma-carcinoma sequence. On the other hand, they are not associated with "de novo" adenocarcinoma. Carcinoma arising from flat adenoma is accompanied by a marker stage II abnormality but only a slight increase in proliferation. The complex proliferation abnormalities occurring in the early stages of intestinal carcinogenesis, therefore, can be divided into elemental alterations for use of separate biomarkers of different levels of the risk of cancer.

Colon Markers-III: Differentiation Markers

WA 012 LECTIN REACTIVITY IN PREMALIGNANT COLORECTAL EPITHELIUM,

C. Richard Boland, Maria Martin, Emily Boohaker, Judy Poore, and Irwin J. Goldstein, Research Service, VAMC, and Depts of Medicine and Biological Chemistry, Univ of Michigan School of Medicine, Ann Arbor, MI.

Colorectal neoplasia is a process that develops gradually and is accompanied by changes in proliferative capacity, genetic structure, and glycoconjugate expression which can be measured using lectins.

In the normal colon, cell differentiation occurs in a gradient from the base to the top of the crypt. Mature goblet cells in the upper portion of the crypt secrete glycoconjugates preferentially bound by the lectins DBA and SBA (indicating terminal α-GalNAc residues). In the lower portion of the crypt, goblet cell mucin is bound by RCA_1 and BPA (indicating terminal β-Gal residues). ACA binds a cytoplasmic glycoconjugate in cells at the base of the normal crypt, whereas PNA does not, suggesting the presence of a substituted (or cryptic) T-antigen. Some variation in these patterns of expression is seen between proximal and distal colon. In small adenomas with low grades of dysplasia, increased proliferation can be found, and generalized labeling throughout the polyp with ACA occurs. Labeling with PNA first occurs in larger adenomas with higher degrees of dysplasia, and always occurs in foci of cancer and the surrounding adenomatous epithelium. The percentage of glands in adenomatous polyps labeled by PNA is proportional to the size of the polyp. SNA (which recognizes α2,6-linked sialic acid residues) binds at low levels in normal colonic epithelium, and labeling progressively increases in larger adenomas. During abnormal proliferation (such as in FAP), a glycoconjugate bound by ACA becomes expressed in the upper portion of normal-appearing colonic crypts, and also binds goblet cell mucin. During progressive neoplasia, binding by PNA and SNA to secreted glycoconjugates increases, suggesting the expression of an α2,6-sialylated T-antigen, which may be a different structure than that bound by ACA in the normal colon. PNA labels glycoconjugates secreted by most cancers, but binding diminishes in metastatic tumors and those primary tumors that give rise to metastases.

Neoplastic progression that occurs in the setting of ulcerative colitis shows similar changes in glycoconjugate expression. DBA binding diminishes in colitic epithelium (independent of the presence of inflammation), and PNA binding is found in the supranuclear (Golgi) region of epithelial cells. PNA first binds to secreted glycoconjugates in the setting of high-grade dysplasia and in carcinomas. Of interest, the appearance of PNA binding in the adenoma-carcinoma sequence and in the dysplasia-carcinoma sequence in ulcerative colitis both occur at the point in neoplastic progression where *ras* gene mutations first occur. Additional correlation between genetic lesions and changes in glycoconjugate expression that occur during tumor progression may help provide appropriate intermediate markers of cancer in the colon.

WA 013 CYTOSKELETON AND OTHER DIFFERENTIATION ANTIGENS, Samuel B. Ho, Dept. of Gastroenterology (111-D), VA Med Center and Dept. of Medicine, University of Minnesota, 1 Veterans Drive, Minneapolis, MN 55417.

Differentiation of intestinal epithelial cells involves a complex process of inhibition of cell division, establishment of cell polarity and commitment to cell lineage. Cell polarization involves formation of junctional complexes, organization of cytoskeletal proteins, and targeting of lipids and proteins to specific membrane domains. Many cytoskeletal and cell lineage markers of normal intestinal differentiation are preserved in preneoplastic and neoplastic colon, and only are lost in the most poorly differentiated carcinomas. These include brush border microvillar proteins, desmosomal plaque proteins, and cytokeratin intermediate filaments. For example, cytokeratin intermediate filaments are a polygenic family of 19 proteins ranging from 40 to 68 kD and are found in epithelial-derived tissues. Using immunoelectrophoresis and immunohistochemical methods, we found that the type of cytokeratin proteins normally found in colonic epithelium was preserved in cell lines from colon cancers and adenomas. In addition, we observed similar crypt-surface distribution of cytokeratins in preneoplastic colon from patients with familial polyposis, colon cancers and inflammatory bowel disease compared with autopsy controls. However, other cytoskeletal and cell lineage markers demonstrate altered polarity in preneoplastic and neoplastic colon. Normal apical polarization of brush border cytoskeletal proteins (actin, villin, fodrin), cell adhesion molecules (CEA) and mucin vacuoles are disrupted in adenomas and cancers, resulting in diffuse cytoplasmic and basolateral expression. Although not found in normal colonic microvilli, expression of brush border enzymes, such as sucrase-isomaltase, occurs in colon cancers, adenomas, hyperplastic polyps, and normal-appearing transitional mucosa. Molecular mechanisms responsible for altered cell polarity remain unclear, however altered protein phosphorylation may play a role. Undifferentiated crypt cells have increased protein kinase activities and phosphotyrosine-containing proteins compared with differentiated villous cells. Furthermore, the phosphorylation status of cytoskeletal and junctional complex proteins appears to influence their solubility and interactive properties, which may result in altered cell polarity. Further characterization of cytoskeletal and associated phosphorylated proteins may allow for improved detection of preneoplastic conditions.

WA 014 BLOOD GROUP-RELATED CARBOHYDRATE ANTIGEN EXPRESSION IN MALIGNANT AND PREMALIGNANT COLONIC NEOPLASMS, Steven H. Itzkowitz, Shunichiro Ogata, Asher Kornbluth, and Anli Chen. Gastrointestinal Research Laboratory, Dept. of Medicine, Mount Sinai School of Medicine, New York, NY 10029.

Cell surface glycoconjugates of colonic epithelial cells carry certain carbohydrate antigens related to blood group substances. During the progression to malignancy, these oligosaccharide immunodeterminants undergo two major types of alterations: (1) neosynthesis of novel epitopes, usually by a process of oligosaccharide elongation; and (2) incomplete glycosylation, resulting in truncated oligosaccharides with exposure of antigens that are normally cryptic. As an example of neosynthesis, the Le^x antigen in its simple trisaccharide form is frequently expressed by a variety of normal, benign, premalignant and malignant colonic tissues, but the synthesis of this antigen on longer oligosaccharides is restricted to malignant and premalignant lesions.

Other carbohydrate antigens, Tn,T, and sialosyl-Tn (STn), are not expressed by normal colonocytes but their exposure in premalignant and malignant colonic neoplasms is indicative of incomplete glycosylation. The STn antigen is of interest because of its high sensitivity (87-90%) and specificity (95-100%) for colon cancer tissues. In addition, hyperplastic polyps rarely express STn antigen whereas 50% of adenomatous (premalignant) polyps express this antigen, especially those with greatest malignant potential. Furthermore, in chronic ulcerative colitis (CUC) with dysplasia, STn antigen expression is increased in accordance with higher degrees of dysplasia. Finally, expression of STn by colon cancer cells was associated with a poor prognosis independent of tumor stage, histologic type, or ploidy status. These observations invoke carbohydrate tumor-associated antigens as potentially useful diagnostic and prognostic markers of colonic neoplasia.

Whether any of these antigens may be useful as intermediate endpoint markers for colonic neoplasia is not known. As a surrogate model, we recently examined STn expression in serial, histologically normal biopsies from individuals undergoing yearly colonoscopic surveillance for dysplasia in CUC. Our preliminary findings revealed that all six CUC patients who eventually developed cancer or high-grade dysplasia (HGD) had STn antigen expression in normal mucosa from the same colonic site 1-9 years (mean 4.2 yr) earlier. In contrast, of 8 matched controls who have not yet developed cancer or HGD, only 3 (38%) expressed STn in prior surveillance biopsies. By inference, then, specific carbohydrate-associated antigens may be useful intermediate endpoint markers for sporadic colonic neoplasms.

WA 015 ALTERED GLYCOSYLATION IN NEOPLASIA, Young S. Kim, GI Res Lab (151M2), VA Med Center & Dept. of Med, Univ. of California, 4150 Clement St., S.F., CA 94121

Many phenotypic markers for malignancy altered carbohydrates. Cancer associated carbohydrate antigens can reside either on N-glycosidic or O-glycosidic (mucin) glycoproteins or on glycolipids. Although much work has been carried out on glycolipids of cancer cells, recent studies indicate that considerable alteration in carbohydrate structures occur in mucin glycoproteins in epithelial cancer cells. Recent cloning and sequencing of cDNAs for two distinct intestinal mucin and of one membrane associated mucin-like glycoprotein indicate that apomucin peptides consist of repetitive tandem repeat segments flanked by non-repetitive unique segments. The repetitive segments are much more heavily glycosylated with O-linked oligosaccharides than the unique segments. With malignant transformation, the tandem repeats are more sparsely glycosylatedthan in normal mucins. Two major changes occur in the carbohydrate side chains of mucin glycoproteins in colon cancer cells. These are: 1) incomplete glycosylation with exposure of normally cryptic inner core sugars such as Tn, sialyl Tn and T antigens, or exposure of protein backbone antigens that are normally masked by heavy glycosylation. 2) modification of existing structures in the peripheral and backbone region of oligosaccharide side chains resulting in novel epitopes such as extended type 2 chain, Le^x, Le^y, sialyl Le^x, or polyfucosylated Le^x and Le^y antigens, and increased expression of carbohydrate antigens (e.g. Le^x) that are normally expressed at a low level. Some of these markers have already been found to be useful as prognostic indicators. Furthermore, they may serve as premalignant markers, since the expression of these cancer associated mucin antigens appear to correlate with the parameters of malignant potential in adenomatous polyps, such as the size, histologic types and degree of dysplasia. Although expression of some carbohydrate antigens are also correlated with proliferative activity of normal colonocytes, whether any of these antigenic alterations can serve as intermediate end point markers in preneoplastic colon remains to be elucidated.

Colon Markers-IV: Genetic Markers

WA 016 ABNORMAL DNA CONTENT AS A BIOMARKER OF LARGE BOWEL CANCER RISK AND PROGNOSIS, Dennis J. Ahnen, Department of Medicine, Denver Department of Veteran's Affairs Medical Center and The University of Colorado School of Medicine, Denver, CO, 80220.

The DNA content of cells can be readily measured by incubating the cell nuclei with a fluorescent dye that binds stoichiometrically to DNA and measuring the intensity of fluorescence of each nucleus by flow cytometry. This technique can estimate the percentage of cells in each phase of the cell cycle and detect cell populations with an abnormal amount of DNA (termed aneuploid cell populations). Flow cytometric analysis of DNA content can be performed on fixed-paraffin embedded tissue but it is more reliable when fresh tissue is analysed (small or near-diploid aneuploid populations are more likely to be missed in paraffin sections). Differences is tissue preparation and the software used to analyse the histograms can result in substantial differences in the interpretation of DNA histograms. Aneuploid cell populations can be detected in about 60% of established large bowel cancers. In general patients with aneuploid large bowel cancers have a poorer prognosis than those with diploid tumors. Some studies report that the proliferative rate (as assessed by the % of cells in S and G2/M phases of the cell cycle) of the tumor cells is also a prognostic factor and it has been suggested that the DNA index (the amount of DNA in the aneuploid population) may be related to prognosis. Aneuploid cell populations can also be detected in premalignant colonic mucosa. About 20% of colonic adenomas contain aneuploid cell populations and large patches of aneuploidy can be frequently (80%) detected in the non-neoplastic as well as the neoplastic mucosa of patients with Chronic Ulcerative Colitis (CUC) associated colon cancer. Patients with CUC and dysplasia (but no cancer) are found to have aneuploid colonocyte cell populations more frequently than those without dysplasia. Some patients with long standing CUC have aneuploid colonocyte cell populations in the absence of any histologic evidence of dysplasia or cancer. Whether the presence of aneuploidy in these preneoplastic settings is predictive of an increased subsequent cancer risk is yet to be determined.

WA 017 MODULATION OF GENE EXPRESSION AS A BIOMARKER IN COLON, Leonard H. Augenlicht and Barbara G. Heerdt, Albert Einstein Cancer Center, Bronx, New York 10467

Computer driven scanning and image processing methodology has demonstrated that genetic inheritance of risk for colorectal cancer in familial polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC) families is associated with highly pleiotropic affects on patterns of gene expression in the flat colonic mucosa. Among a panel of cloned sequences identified which characterize genetic risk, one is the mitochondrial (mt) gene encoding subunit 3 of cytochrome oxidase (COXIII). Expression of COXIII decreased in progression of, and risk for, colonic tumors in vivo. Further, metabolizable, unbranched, short-chain fatty acids (SCFAs) elevated expression of mtCOXIII, as well as mtCOXI, in HT29 cells and also elevated mtCOX enzymatic activity. However, expression of nuclear encoded COX subunits were unaffected. These changes may be related to documented alterations in mitochondria strucuture and function in transformed colonic epithelial cells.

SCFAs produced by fermentation of fiber by colonic microflora are the principle energy source for normal colonic epithelial cells, and SCFAs also induce a more differentiated phenotype both in vitro and in vivo. Therefore, a mechanistic link may exist between molecular events in inherited risk and a dietary factor (fiber) which may modulate such risk.

In a preliminary intervention trial in collaboration with M. Lipkin, high risk HNPCC patients received daily supplements of 1500 mg CaCO3 per day, which may be protective for development of colorectal tumors. Elevations in COXIII expression were seen in 7 of 12 patients within the first 7 months, followed by complex changes in expression of this sequence.

WA 018 SUPPRESSOR GENE ALTERATIONS IN THE ADENOMA-CARCINOMA SEQUENCE, Kathleen R. Cho[1,2] and Bert Vogelstein[1], the Johns Hopkins Oncology Center (1), 424 North Bond Street, Baltimore, MD 21231 and Department of Pathology (2), The Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21205.

Tumorigenesis is thought to be a multistep process in which genetic alterations accumulate to bring abut the neoplastic phenotype. Colorectal tumors appear to arise as a result of the mutational activation of oncogenes coupled with the inactivation of several tumor suppressor genes.

We have found frequent allelic deletions of specific portions of chromosomes 5,17, and 18 which presumably harbor suppressor genes. The target of allelic loss events on chromosome 17 has been shown to be the p53 gene which is frequently mutated not only in colon cancer but in several other tumor types as well. Candidate suppressor genes have also recently been identified on chromosomes 18 and 5. The DCC gene on chromosome 18q encodes a protein with significant sequence similarity to neural cell adhesion molecules and other related cell surface glycoproteins. Alterations of this gene may interfere with normal cell growth and differentiation by disrupting cell-cell or cell-substrate interactions. Two genes (MCC and APC) on chromosome 5q have also recently been identified and partially cloned. These genes are located in a region tightly linked to familial adenomatous polyposis (FAP). While MCC mutations have been found only in sporadic colon tumors, APC mutations have been identified in sporadic tumors as well as the germline of patients with FAP. Studies are currently in progress to increase our understanding of how alterations of these genes affect colorectal tumor cell growth.

WA 019 ONCOGENES, Geoffrey M. Cooper, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115

Oncogenes are mutant forms of normal cell genes (proto-oncogenes) which contribute to the process of neoplastic transformation. Over sixty different cellular oncogenes/proto-oncogenes have been characterized. Many proto-oncogenes encode proteins which function in signal transduction pathways leading to normal cell proliferation. Their gene products include growth factors, growth factor receptors, plasma membrane-associated protein-tyrosine kinases and guanine nucleotide binding proteins, cytoplasmic protein-serine/threonine kinases, and transcriptional regulatory proteins. Oncogenes are formed as a result of mutations or DNA rearrangements that lead either to abnormal expression of a proto-oncogene or to abnormal function of its gene product. The resulting elevation in either the amount or activity of the oncogene proteins leads to abnormal cell proliferation, thus contributing to the development of malignancy.

About fifteen distinct oncogenes are currently known to be regularly involved in human neoplasms. These include oncogenes which appear to play a role in early stages of tumor formation (e.g., ras*K* in colorectal carcinomas), as well as oncogenes which appear to be involved in later stages of tumor progression (e.g., N-*myc* in neuroblastomas). The possibility of utilizing such oncogenes as markers of tumor development will be discussed.

Cooper, G.M. 1990. Oncogenes. Jones and Bartlett Publishers, Boston, MA.

WA 020 THE DEVELOPMENT OF ANEUPLOIDY DURING TUMORIGENESIS, Walter N. Hittelman, Sun Y. Kim, Jae Y. Ro, Waun K. Hong, and Jin S. Lee, The University of Texas M.D. Anderson Cancer Center, Houston.

The process of malignant development is thought to be a multistep process where an accumulation of specific genetic events results in dysregulation of proliferation and differentiation in the target tissue. The importance of genetic instability in tumor development is underscored by four types of findings. First, individuals with chromosome breakage syndromes are found to have a higher risk to tumor development. Second, most active carcinogens are also known to induce mutations, deletions, and chromosome aberrations. Third, most human tumors, especially solid tumors, are found to exhibit multiple chromosome changes by the time of tumor development. And fourth, human solid tumors are often found to exhibit considerable karyotypic variation evolving from a common set of clonal abnormalities. Recent studies from our laboratory, using the technique of premature chromosome condensation (PCC) to visualize chromosome changes, have shown that apparently normal lung tissue obtained at the time of lung tumor resection exhibits significant chromosomal changes, sometimes similar in extent (but not necessarily identical) to that found in the tumor. Similarly, PCC studies in a hamster cheek pouch tumorigenesis system shows increased accumulation of chromosomal alterations during continued carcinogen exposure. More recently, using the technique of in situ hybridization with chromosome-specific DNA probes, we have been able to demonstrate the development of aneuploidy in premalignant lesions. Moreover, the degree of apparent aneuploidy increases with histological grade and is associated with increased dysregulation of proliferation. These results would suggest that the development of aneuploidy might be used as a biomarker with regard to the degree of progression along a multistep tumorigenesis pathway. In early stages, the development of random chromosome changes might reflect ongoing genetic instability, due either to carcinogenic exposure or inherent instability. A subsequent change might involve the selective outgrowth of aneuploid clones with proliferative advantage, differentiative dysregulation, or defective cell loss. Finally, the accumulation of a sufficient number of particular chromosomal changes would be associated with the progression to malignancy. Since these changes can now be assayed directly in apparently normal or premalignant tissues, the degree of genetic change might serve as a marker for the degree of risk of progression to malignancy.

Markers for Other Target Sites

WA 021 PROSTATIC INTRAEPITHELIAL NEOPLASIA: A PREMALIGNANT LESION, Michael K Brawer,Department of Urology RL-10, University of Washington, Seattle, WA 98195

Putative premalignant changes in the prostate have been recognized for a number of years. A variety of synonyms have been given to the most commonly described lesion, characterized by proliferation and dysplasia of the normal two cell layers lining prostatic acini in ductules. Prostatic intraepithelial neoplasia (PIN) is the term most commonly used to describe this entity.

Requirements for a lesion to be considered premalignant include morphologic features similar to invasive carcinoma (CA), a spatial association with CA micro invasive cancer arising from the lesion, and the frequency, severity and extent of the premalignant change should occur to a greater degree in organs harboring CA. Finally, and most definitively, progression from the premalignant lesion into CA should be observed over time. PIN fulfills all but the last of these requirements.

High grade PIN is cytologically indistinguishable from prostate carcinoma (CAP). The major differentiating feature between PIN and CAP is the presence, although frequent disruption, of the basal cell layer in the former. We have studied this using high molecular weight cytokeratin and demonstrated a correlation between PIN grade and the percent disruption of the basal cell layer. Phenotypically the cells making up PIN are similar to those of CAP. We have utilized a variety of markers including cytokeratins, vimentin and the lectin Ulex euroapaeus. Additionally we and others have noted decreasing PIN immunoreactivity with antibodies directing against prostate specific antigen and prostatic acid phosphatase. Other investigators have noted additional phenotypic similarities between PIN and CAP including the ABH and Lewis antigens. PIN incidence and grade correlate well with the presence of CAP elsewhere in the prostate. In fact we have noted PIN in all cases of peripheral zone CAP in which radical prostatectomy specimens were available for review.

The definitive requirement for a premalignant lesion is its observation to undergo invasion over time. This has not been proven with PIN because it is impossible to serially biopsy the same acinar-ductule system on separate occasions.

The clinical importance of PIN follows from three primary observations. We and others have demonstrated the PIN may be associated with elevation of serum PSA level. PIN may appear on transrectal ultrasound of the prostate (the optimum imaging modality for this organ) to give rise to a hypoechoic lesion similar to the most common presentation for CAP. Finally we have noted that when PIN is noted on a prostate biopsy there is an exceedingly high incidence of finding CAP on a repeat biopsy.

It would thus appear that PIN represents a major premalignant lesion in the human prostate. The potential for strategies of chemo prevention to inhibit further transformation or progression of PIN into invasive carcinoma seems tenable and worthy of further investigation.

WA 022 MARKERS FOR PREVENTION OF CERVIX (C), BONE MARROW (BM), AND CUTANEOUS (CU) CANCERS (CA)

Frank L. Meyskens, Jr., Department of Medicine and Clinical Cancer Center, University of California Irvine, Orange, California 92668

Early CA formation in C is monitored by serial colposcopy, cytology, and biopsy. The histologic features of cervical intraepithelial neoplasia (CIN) are known and differentiation and proliferation features characterized. The role of certain human papilloma virus subtypes (6,8,16,18) in the genesis of CIN is clear. Modulation by chemopreventive agents should be informative.

The problem of BM disease after treatment for lymphoma is a serious one, with up to 10-12% of cured patients subsequently developing leukemia. Serial monitoring of BM for preclinical detection of the expression of abnormal mRNA should be possible.

Markers for two major different types of cutaneous CA should be considered: non-melanoma (NM) and melanoma (M) CU cancers (SC). NMSC represent an array of abnormalities, ranging from frequent CU CA in patients with xeroderma pigmentosum to extensive actinic damage and facial cancers in sunexposed elders. Different clinical and histologic precursors to NMSC are identifiable. NMSC represents a "field defect" of the CU environment, a number of other parameters can also be monitored in non-involved skin. NMSC is largely a product of ultraviolet light (UVL), a process that has been characterized at the molecular and immunological level. The following features should be monitored: cellular immunologic composition (esp. T-suppressor cells), DNA repair, and ras oncogenes, especially c-Ha-ras gene. The risk for M is related to the number and types of moles and intense exposure to UVL.

Abnormal moles can be clinically and histologically assessed. Particularly valuable has been delineation of the stepwise process by which melanocytes (MC) are transformed to M: a) gain of HLA type II and loss of type I antigens; b) clonal cytogenetic abnormalities; c) loss of dependence on TPA for growth; d) loss of protein kinase C-β isotype; and e) dysregulation of the jun family of genes. I will describe this latter model in detail as well as our early attempt to perturb MC in vitro with UVL and to measure its effects on molecular parameters.

WA 023 CANDIDATE BIOMARKERS FOR APPLICATION AS INTERMEDIATE END POINTS OF LUNG CARCINOGENESIS, James Mulshine, Biomarkers and Prevention Research Branch, Division of Cancer Prevention and Control, NCI,NIH,Bethesda, MD, 20892.

The need for validated intermediate end point markers to facilitate lung cancer chemointervention research is compelling. Three major classes of lung markers are relevant for this application. Since lung cancer includes four distinct histologies, markers that map degrees of histologic differentiation are important. Many of the markers for squamous differentiation overlap with the candidates for application in the study of head and neck cancer. Production of tissue-specific cell products especially for surfactant or CEA is of interest, because the gene structure is known and many differentiation-related polymorphism exist. This strategy would be useful for adenomatous type tissue. A second type of marker is the broad group of differentiation markers. The carbohydrate or blood group-like antigens comprise a representative example. Carbohydrate structures are expressed in a specific sequence during fetal processes and this sequence appears to reverse with the development of a cancer. Retrodifferentiation of specific differentiation markers is the basis of a major effort to effect earlier lung cancer detection using sputum immunocytochemistry. The final class includes markers which effect either positive or negative aspects of growth. Candidates in this area include growth factor or their receptor, or genes that regulate growth. If the intermediate point marker reflect tumor biology and that biology is in the casual path of tumor progression, serial observation of that parameter should indicate the success of the intervention. In all three of these examples the clinical material to be analyzed is lung tissue which could be sputum specimens, bronchial biopsies or resected lung tissue. Systematic analysis of these markers in context of intervention trials is required to validate the utility of these markers. Long term clinical follow-up will demonstrate the degree of concordance between biomarkers with more traditional clinical trial end points and will establish if such tools can play a role in catalyzing the rate of prevention research.

WA 024 INDICATORS OF INCREASED BREAST CANCER RISK,

David L. Page[1], William D. Dupont[2], Vanderbilt University Medical Center; Department of Pathology[1] and Preventive Medicine, Statistics[2]; Nashville, Tennessee 37232.

Specific atypical histological patterns of epithelial hyperplasia (AH) indicate a medically relevant risk of breast cancer development in 5-10% of women with otherwise benign biopsies. This risk is about four times that of similar women, i.e. of the same age and at risk followed for the same length of time. This time interval is probably best limited to 10-15 years, as longer periods of prediction are not supported by currently available data. It is our experience, particularly with older women, that these relative risks are not stable and will fall 10-15 years after detection.

Absolute risk for AH is about 10% in 10-15 years. In general terms, we do not feel that breast cancer risk should be extended beyond that length of time because the stability of risk with time is unproven.

There is such a strong interaction with family history and AH that it is relevant to consider women with atypical hyperplasia who have a positive family history of breast cancer separately from those who do not. The absolute risk of breast cancer development in women with atypical hyperplasia without a family history was 8% in 10 years, whereas those with a positive family history experienced a risk of about 25% at 15 years. This interaction has also been observed in other recent studies.

Low replacement doses of conjugated estrogen after menopause do not further relevant risk beyond that identified by histology. In our cohort of over 10,000 women who underwent benign breast biopsy in Nashville, TN, we found no association between proliferative breast disease without atypia and a first-degree family history of breast cancer; the prevalence of these lesions was 27% and 29% in women with and without such a history, respectively. Women with this family history did, however, have a higher prevalence of AH than did women without this history (4.8% versus 3.9%, respectively, $P = 0.02$). It would appear that these histologic lesions are not due to an estrogen effect, but are an unrelated phenomenon.

WA 025 PRECANCEROUS LESIONS OF THE HUMAN ESOPHAGUS: MULTIPARAMETER STUDY OF ESOPHAGEAL BIOPSIES FROM A HIGH-RISK POPULATION IN LINXIAN,CHINA, Kan Yang, Martin Lipkin. Irving Weinstein Laboratory for Gastrointestinal Cancer Prevention, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Two hundred and twenty-one esophageal biopsies were taken from subjects with cytologic hyperplasia in Linxian, a high risk area for esophageal carcinoma in China. The study included morphology, morphometry, tritiated thymidine incorporation and immunohistochemistry. The pathologic abnormalities included a spectrum of morphologic changes, grouped as follows: (1) normal/near normal (NN) (10.0%); (2) basal cell hyperplasia 0 (one epithelial layer, BH0) (17.2%); (3) simple hyperplasia (SH) (14.5%); (4) mixed hyperplasia (basal and spinous, MBS) (29%); (5) basal cell hyperplasia 1 (multiple basal cell layers, BH1) (15.8%); (6) dysplasia (D) (7.2%); and (7) non-proliferative lesion (NP) (6.3%). Forty percent of the biopsies had combinations of histologic types. Three growth patterns were seen: flat (F), endopapilloma-like (EP) and spike (SP). F pattern (72.0%) appeared in each group. EP was only seen in BH0, SH, MBS, BH1 and D. The thickness of the epithelium was increased in SH, MBS, and BH1 significantly different from NN, but not BH0 and NP. Number of papillae was not different among the groups studied, and 61 of 127 (48%) of biopsies with single lesions had branched papillae. Elongation of papillae was also seen in SH, MBS, BH1 and D with significant differences compared to NN. Bleeding in the epithelium around papillae was very prevalent in the esophageal specimens studied. Every NP biopsy had bleeding, also present in more than 2/3 of specimens of the other groups. A variety of cellular changes were found in papillary areas especially when bleeding occured. Chronic inflammation was demonstrated in epithelium of 33.6% of biopsies, in 60.9% papillae and in 86.1% of lamina propria areas. ^{3}H thymidine labelling index was highest (0.11) in dysplasia with significant differences compared to all other groups. PCNA labelling index in papillary areas was almost 3 times higher than in flat epithelial areas. Immunohistochemical study of blood group antigen LeY and lectin WGA showed that the cellular membrane component of esophageal squamous epithelium changed before morphologic alterations. These findings provide a hypothesis for the sequence of pathogenetic events leading to esophageal carcinoma, and establish and quantitate intermediate biomarkers in this population for cancer prevention studies.

Intermediate Biomarkers of Precancer

WA 100 NUMERICAL ABERRATIONS IN CHROMOSOME 17 IN BREAST CARCINOMAS DETECTED BY FLUORESCENT *IN SITU* HYBRIDIZATION (FISH), Douglas C. Aziz, Cytometrics, Inc., Division of Specialty Laboratories, Inc., San Diego, CA 92121

Loss of genetic material from the short arm of chromosome 17, specifically from the p53 gene, is associated with a wide variety of human tumors. Early-onset familial breast cancer is linked to a region on the long arm of chromosome 17. Amplification of c-*erb* B2, which is localized to the long arm of chromosome 17, is associated with a poor prognosis in breast cancer. Because of the importance of genetic changes on chromosome 17, we investigated the relationship of loss or gain of chromosome 17 to other prognostic markers in breast cancer. Fifty-seven carcinomas of the breast were examined for numerical aberrations of chromosome 17 by fluorescent *in situ* hybridization (FISH) using chromosome-specific probes to the α-satellite DNA. Our interphase cytogenetic study compared these numeric chromosomal aberrations to other prognostic markers, such as DNA ploidy and %S phase analysis (flow cytometry), c-*erb* B2 oncogene expression (immunohistochemistry) and estrogen and progesterone receptor positivity (immunohistochemistry) . Strikingly, 31% (9/29) DNA diploid cases were monosomy 17 and 21% (6/29) were trisomy 17. The mean and median DNA Index (DI) of the DNA aneuploid cases correlated with the number of chromosome 17s present in the interphase nuclei. The mean and median DI for monosomy, disomy, trisomy, and hypertetrasomy were 1.5 and 1.3, 1.6 and 1.3, 1.9 and 1.7, and 2.1 and 2.1, respectively ($p<0.03$, sign test for disomy and trisomy). The frequency of numeric aberration of chromosome 17 in estrogen and progesterone receptor positivity, c-*erb* B2 overexpression and proliferative fraction (%S-phase) did not differ significantly from the overall frequency in all 57 specimens. FISH can be used successfully to enumerate chromosomes in interphase nuclei in paraffin-embedded tissue. This technique will be particularly useful when numeric aberration data are available for all of the chromosomes. DNA diploid tumors, as measured by flow cytometry, are not truly diploid but contain some chromosome loss or gain. DNA aneuploidy reflects gross chromosomal aberrations and is associated with increased tumor aggressiveness. Detection of numeric aberration of specific chromosomes by FISH should lead to a better understanding of the mechanisms underlying the progression from precancer to cancer.

WA 101 MOLECULAR DOSIMETRY OF AFLATOXINS IN CHEMOPREVENTION TRIALS WITH OLTIPRAZ

M. G. Bolton, P. DeMatos, J. D. Groopman, and T. W. Kensler
Department of Environmental Health Sciences, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205.

Epidemiological studies indicate that three-fourths of human cancers are caused by environmental carcinogens and should therefore be preventable. Traditional methods for risk assessment, however, depend on population-based statistics and thus fail to identify individuals at high risk. The field of molecular dosimetry deals with the development of biomarkers of exposure that correlate with biologically effective doses of toxic species at their target sites. Such biomarkers may be particularly useful as intermediate endpoints in a chemoprevention trial as they permit the quantitative assessment of individual exposure status, provide a more accurate prediction of risk, and thereby improve selection criteria for individual participation in an intervention trial. More importantly, these biomarkers provide insight into the mechanisms of chemical carcinogenesis and thus provide a more rational basis for chemoprevention strategies. Aflatoxin B_1 (AFB_1) is a known rat hepatocarcinogen and a putative human carcinogen. AFB_1 is converted to an 8,9-epoxide which can interact covalently with nucleophilic centers in DNA, RNA, and protein. Two of the major macromolecular adducts that have been identified are the AFB-N^7-guanine adduct in DNA and a lysine adduct in serum albumin. Overall, there is an excellent correlation between the dose of AFB_1 and levels of epoxide-derived adducts: hepatic AFB-DNA adducts, AFB-N^7-guanine adducts excreted into the urine, and serum AFB-albumin adducts. Thus, these metabolites represent biomarkers of exposure and are likely related to the underlying mechanisms by which aflatoxin exerts its carcinogenic effect. Apart from binding to macromolecules, the epoxide can undergo detoxication through hydrolysis to AFB-diol or conjugation with glutathione. Oltipraz, a potent inducer of electrophile detoxication enzymes such glutathione S-transferases, is a substituted dithiolethione that has been used clinically as an antischistosomal agent. It exerts a dramatic protective effect against AFB_1-induced hepatocarcinogenesis in the rat. Treatment with oltipraz also produces marked reductions in hepatic AFB-DNA adducts as well as in the readily accessible biomarkers - urinary AFB-N^7-guanine adducts, and serum AFB-albumin adducts. Thus, these biomarkers of exposure are predictably modulated by oltipraz and provide rational short-term endpoints for evaluating chemoprevention trials with oltipraz in humans at high risk for aflatoxin ingestion and primary hepatocellular cancer.

WA 102 LIPOPHILIC MICRONUTRIENTS IN HUMAN PLASMA INDICATE OXIDATIVE DAMAGE, Adrian Franke, Terry Shimamoto, Sara Lumeng and Bob Cooney, Cancer Center of Hawaii, 1236 Lauhala St., Honolulu HI 96813.

Free radicals, especially oxygen radicals are believed to play a vital role in the complex course of carcinogenesis. Antioxidants have been shown to inhibit this multistep neoplastic process and their consumption has been associated with reduced cancer incidence rates in humans. Low serum beta-carotene has been identified as a marker of risk for cervical dysplasia and cancer. We investigated the levels of diverse dietary antioxidants in humans and their role as chemopreventive agents. Carotenoids, retinoids and tocopherols from human plasma are generally determined by HPLC analysis after ethanol precipitation of proteins and hexane extraction. While tocopherols show good recovery with variations in the extraction procedure, many factors influence the extraction efficiency of carotenoids and retinol. We report the optimal conditions to standardize this procedure in order to achieve comparable interlaboratory results. We describe a fast analytical method with good recovery (98%) and excellent reproducibility (s_{rel}<4%) using two internal standards, one for the carotenoid/retinol group and one for the tocopherol group. Both groups are analyzed simultaneously in one HPLC run. In this way we can quantify 30-50 plasma samples in duplicate per day. Using this method we describe plasma levels and their temporal variation for alpha- and beta-carotene, lycopene, alpha- and beta-cryptoxanthin, lutein/ zeaxanthin, retinol and alpha-, gamma- and delta-tocopherol in a human population. Correlations between plasma levels of these diverse antioxidants and lipid peroxide levels are discussed.

WA 103 METALLOPROTEINASE ACTIVITY IN NORMAL AND MALIGNANT MAMMARY GLAND DEVELOPMENT.

Robert Friis, Li Feng and Robert Strange, Laboratory for clinical and experimental Research, University of Berne, Tiefenaustr. 120, CH 3004 Berne, Switzerland.

We have recently studied changes in gene expression characteristic of mammary involution, the phase in which the mammary gland restructures after lactation. The attendant tissue re-modelling involves the programmed cell death of more than 70% of the elaborate ductal epithelium needed for milk production. We have cloned and sequenced a metalloproteinase (designated clone 24) related to Transin-1 which is almost uniquely expressed in the process of mammary involution; Northern blots failed to exhibit significant expression in any organ other than involuting mammary gland and salivary gland. The tissue inhibitor of metallo-proteinases, TIMP-1, is down-regulated during in-volution, at the time when clone 24 gene expression peaks.

Here we report the massive and coordinate over-expression of clone 24 and TIMP-1 in mammary gland tumors produced in H-ras transgenic mice, but not in mice carrying transgenic myc construct-induced tumors.

WA 104 YAC CLONES COMPRISING 5.5Mb OF DNA AT THE FAP LOCUS (5q21) IN SIX CONTIGS ALLOW LOCALISATION OF POLYMORPHIC MARKERS AND CANDIDATE TUMOUR SUPPRESSOR GENES, Philip J. Hedge*, Douglas McKechnie*, Andrew Gammack*, John Riley*, Jon Ellston*, Kenneth Kinzler@, Bert Vogelstein@, Yusuke Nakamura+ and Alexander F. Markham*. * ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK, @ Johns Hopkins School of Medicine, Baltimore, MD 21231 and + Cancer Institute, Toshima-Ku, Tokyo, Japan.

Evidence from loss of heterozygosity in sporadic colorectal tumours and genetic linkage studies in Familial Adenomatous Polyposis (FAP) families has previously demonstrated the presence of a putative tumour suppressor gene, involved in both sporadic and hereditary colon cancer, on chromosome 5q21. The markers YN5.48 and YN5.64 are known to be markers close to and flanking the FAP gene and recent data obtained by characterising small deletions in tumours and FAP kindreds indicate that the anonymous markers L5.79 and EF5.44 are internal to these. We have constructed six contigs of Yeast Artificial Chromosomes (YACs), totalling approximately 5.5Mb of genomic DNA, from between YN5.48 and YN5.64. One contig of 1.6Mb spans the two markers L5.79 and EF5.44 and is therefore likely to contain the gene for FAP. This contig contains the recently identified MCC (Mutated in Colorectal Cancer) and APC genes. By using one of the YACs to screen a cDNA library we have demonstrated that there are several other genes within 200kb of the MCC gene and as such these are also commonly deleted in colorectal tumours. The location of these genes, polymorphic markers and other sequence tagged sites will be described with respect to the YAC contigs.

WA 105 GENETIC MARKERS IN THE MALIGNANT TRANSFORMATION OF PROSTATIC CELLS, Alvin Y. Liu and Barbara A. Abraham, Cytometrics, San Diego, CA 92121.

cDNA libraries were generated for a normal human prostate, [N]cDNA, and a prostate adenocarcinoma, [C]cDNA. The cancer prostate was characterized by well-differentiated, diploid prostatic cells with no evidence of metastasis. It is of low Gleason grade (1+2). Subtraction libraries of normal minus cancer, [N-C], and cancer minus normal, [C-N], were generated. By screening against the [N] and [C] libraries three candidate [N]-specific clones, pN39a, pN44 and pN141f, were isolated. These clones were not detected in the [C] library. pN44 is 476 bp long and encodes a full-length gene for the human prostatic secretory protein, PSP_{94}[1]. The concentrartion of PSP_{94} in the seminal plasma is about 1 mg/ml[2]; it is also found in high concentration in tracheal secretion[3]. Its concentration, however, decreases in adenocarcinomatous prostate[2], which correlates with our subtraction result. Furthermore, no PSP_{94} transcript was detected in two cell lines, PC3 and DU145, derived from prostatic metastases, though the gene was not deleted[4]. pN39a and pN141f are novel cDNAs. Again, no pN39a transcript was detected in PC3 or DU145; and low level of pN141f transcript was found in both lines. We are interested in how these genes are silenced when normal prostatic cells become malignant as a possible early event in transformation. Preliminary analysis of the [C-N] library yielded three clones. One, pC29, showed a quantitative difference in the level of expression (denoted by the frequency of clones in the library as indicated by the intensity of the hybridization signal). The level was higher in [C]. pC29 encodes the human mitochondrial hinge protein. The gene is nuclear encoded, and it was shown to be down-regulated in HL60 cells when these cells were induced to differentiate by TPA[5]. pC29 appeared to be up-regulated when normal prostatic cells became transformed. Two other clones, pC18 and pC35, are novel cDNAs, and are potentially useful markers to assay for in the detection of cancer cells.

References: **1.** Mbikay, M. et al. DNA 6 (1987) 23. **2.** Dubé, J.Y. et al. J. Androl. 8 (1987) 182. **3.** Weiber, H. et al. Am. J. Pathol. 137 (1990) 593. **4.** Brar, A. et al. J. Androl. 9 (1988) 253. **5.** Ohta, S. et al. FEBS Lett. 226 (1987) 171.

WA 106 ELEVATED MITOCHONDRIAL GENE EXPRESSION OF HT-29 HUMAN COLON ADENOCARCINOMA CELLS DIFFERENTIATED IN PRESENCE OF TREHALOSE, V.L.Seligy, X.Lu, T.Walker, and J.P. MacManus, Molecular Cell Biology Group, Institute of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, K1A 0R6, Canada

HT-29 is a poorly differentiated, multi-potent tumour cell line which can be induced to differentiate into enterocyte-like goblet and absorptive cell phenotypes by various substances other than glucose. At present it is not clear to what extent carbon-sources such as galactose and absence of glucose (glucose-deprivation) play a role in mediating the differentiation response of *HT*-29. Replacement of glucose by a disaccharide derivative, trehalose (*α-D-glucopyranosyl-α-D-glucopyranoside*) in *HT*-29 cultures was found to depress growth and promote mucin-producing, goblet-like cell maturation. Sequence analysis of seven cDNA clones whose RNA templates were expressed at elevated levels in cells grown in trehalose-containing medium indicated that they were found in 6 different mitochondrial (*Mt*) genes of the *Hela Mihsxx* sequence. Northern analysis and quantitation by use of a computing-laser densitometer revealed relative differences in amounts of processed *Mt* RNA, ranging in magnitudes of 3 (*16s RNA*) to 23 (*NADH dehydrogenase subunit* 4, *ND*4) based on levels of nuclear encoded elongation factor, *EF*1α mRNA. Levels of *Mt* DNA copy were shown to be unaffected. The *ND*4 DNA was a useful probe for monitoring status of *HT*-29 cell differentiation states in comparison to other cell lines. Levels of *ND*4 RNA were highest in cells in medium containing trehalose, followed by sodium butyrate $\geq$ galactose > no sugar > cellobiose > glucose. These results have particular significance for the use of trehalose and *HT*-29 cells in models for clinical colorectal cancer studies and fundamental studies on the roles of glycolysis/oxidative phosphorylation in cell differentiation and transformation.

WA 107 ALTERATIONS IN COLONIC EPITHELIAL CELL PROLIFERATION IN A RAT MODEL OF NONGENOTOXIN-INDUCED COLON CARCINOGENESIS, David K. Wilcox and Timothy A. Bertram Human Safety Department, The Procter & Gamble Co. Cincinnati OH. 45239

Qualitative and quantitative alterations in colonic cell proliferation were studied using a nongenotoxic sulfated polysaccharide, poligeenan, which induces colorectal tumors in the F344 rat. Proliferating Cell Nuclear Antigen (PCNA) and thymidine kinase activity (TK) were assayed as markers of colonic cell proliferation. F344 rats were fed basal diets supplemented with the carcinogenic fiber, poligeenan, and the noncarcinogenic fibers, guar and carrageenan, at the 5% level. After 28 days of exposure, the poligeenan-fed rats exhibited a 13-fold increase in the number of PCNA-positive cells in the upper third of the crypt. This increased to 35-fold after 91 days. TK activity in the poligeenan group increased 5 fold within 9 days of exposure, and this level was maintained throughout the 91-day exposure. To examine the ability of the colonic epithelium to recover from exposure to poligeenan, a second set of animals was fed each of the test diets for 64 days followed by a 28-day recovery period on basal diet alone. The poligeenan-treated animals failed to recover. The number of PCNA-positive cells in the upper third of the crypt remained 11-fold above controls. TK activity remained 2-fold above the controls. In contrast, recovery from carrageenan was complete within the 28-day period. No PCNA positive cells were observed at the luminal surface and TK activity returned to basal levels during the 28 day recovery. Guar showed only a transient increase in TK activity. These data suggest that proliferating cells at the luminal surface and loss of the ability to down-regulate proliferation during recovery may be early events in the carcinogenic process.