EXFOLIATED CELLS, BIOACTIVE FOOD COMPONENTS, AND CANCER PREVENTION WORKSHOP

Nutritional Science Research Group
Division of Cancer Prevention
National Cancer Institute

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Welcome and Introduction *Drs. Cindy Davis, Peter Greenwald and John Milner*

Dr. Davis, Chair of the workshop, welcomed participants and thanked them for attending. Dr. Davis explained that the purpose of the workshop was to identify and evaluate the strengths and weaknesses of using exfoliated cells to monitor changes in gene expression, DNA methylation, protein expression, accumulation of bioactive food components, and/or predicting the anticancer response of target tissues in response to essential and nonessential nutrients. The workshop focused on the use of cancer cells exfoliated from the lung, colon, and mammary glands because cancers from these organs account for the majority of cancers in the United States each year, and evidence exists that all three cancers are modified by dietary components. Workshop participants were encouraged to consider new and innovative ways to use exfoliated cells in studies of nutrition and cancer prevention, and were encouraged to create new research collaborations for the future.

Dr. Greenwald, Director of the Division of Cancer Prevention at the National Cancer Institute (NCI), also welcomed attendees and thanked them for their participation. Dr. Greenwald described a need to increase awareness that basic nutritional science is as important a component of cancer research as epidemiology and behavioral science. There is strong evidence that prevention has two major parts: public health awareness (i.e., do not smoke, modify your diet, live a healthy lifestyle) and medical approaches with newer diagnostic techniques (i.e., developing and validating biomarkers and relating them to the modulation of metabolic pathways). Three areas of nutritional science need expanded research. They are:

- Nutritional preemption—how nutrition and healthy lifestyle maintain good health
- Personalized nutritional translation—how individual differences in metabolism affect public health
- Functional food development and delivery—understanding and characterizing how different nutrients work. The food and agriculture industries are already modifying food products to meet production requirements and shelf life limitations. These efforts need to be coordinated with research to ensure that productions and marketing decisions are based on nutritional health research findings.

Dr. Milner, Chief of the Nutritional Science Research Group, also welcomed participants to the workshop. Dr. Milner stated that ongoing research is addressing some of the questions raised by Dr. Greenwald, which are vital to understanding how dietary habits contribute to the 30-35 percent of cancers recognized as diet-related. The sciences of nutrigenetics (the study of how genes affect the bioactivity of food components) and nutrigenomics (how nutrients modify gene expression) are helping unravel the role of nutrition in the cancer process by delineating how nutrients modify genes that promote the development of cancer, and identifying molecular targets in neoplasia. These issues have been addressed in recent Requests For Applications (RFAs). Other useful avenues of research are determining what concentrations of nutritional components are needed for positive gene modification in different individuals and what tissues should be examined to make these determinations; finding the sites of action of various nutrients; and discovering whether significant levels of nutrients are necessary before genes can produce physiological effects and deciding which tissues should be monitored to track these levels. Although this program examined three primary cancer sites, the information and principles it uncovered might have broad implications for other cancers.

OVERVIEW TOPICS

Bioactive Food Components in Cancer Prevention: Limitations of Serum Concentrations as a Predictor of Response

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There is a new paradigm in nutrition research within NCI. Recommendations for preventing and controlling cancer must have a strong scientific basis, and understanding the specific role of nutrients is critical. Scientists are focusing their research on how essential and nonessential nutrients influence genetic pathways and how dietary constituents interact with individual genetic profiles (polymorphisms). The current approach to nutrition research may not be adequate. Clinical, basic, and translational research must work together and interinstitutional and interdisciplinary collaborations will be essential to achieving these new research goals.

Evidence is rapidly accumulating that specific components of food, alone or in combination with one another, have potent effects upon prevention of a wide variety of neoplasms. Bioactive food components in cancer prevention have been studied, and the complexity of the issue is daunting. Reviewing the potential nutrient modifiers of prostate cancer illustrates the complexity, especially given the difficulties in using blood levels to measure their response, their intake, and their actions. Nutrient modifiers being studied for prostate cancer include: allylsulfides, considered the most important potential nutrient modifier; calcium and Vitamin D (the latter causes differentiation and regulates calcium metabolism); epigallocatechin-3-gallate (EGCG), obtained from tea and related compounds (this modifier has been related to prostate cancer prevalence); fatty acids found in fish, which appear to

relate to the decrease in prostate cancer with fish intake; genistein from soy, which has estrogenic activities and appears to be an important agent in prostate cancer prevention; indole-3-carbinol, found in cruciferous vegetables (its metabolites are not themselves potent hormones but influence hormone metabolism); lycopene, found in tomatoes and tomato products; resveratrol, found in grapes and ancient Chinese weeds, has at least eight actions that are protective against cancer and some that are protective against heart disease; selenium, whose importance is supported by basic science and for which translational research is now being done; Vitamin A, whose uptake and binding are being explored; and Vitamin E. Measurement of the serum levels of these agents often provides an insensitive, inaccurate, or misleading index of dietary intake.

Why is it difficult to measure the responses of these nutrient modifiers using blood samples? A good example of the difficulty can be seen by reviewing the metabolism of garlic, calcium, vitamin A, and vitamin E.

Crushing, mincing, and chopping a garlic bulb releases alliin that is then converted into allicin, which is metabolized into a large number of mainly fat-soluble compounds. There are some water-soluble derivatives of allicin, principally s-allyl methylcysteine, which is derived from ?-glutamylcysteine. It would appear that measuring the intake and efficacy of garlic would be achieved by measuring the allicin; but allicin is barely detectable in human blood. It is highly reactive, very unstable, and difficult to measure. There is some question as to whether it is actually absorbed. Attention should focus on the water-soluble derivatives, and their levels should be used to standardize garlic preparations.

Calcium is absorbed, but measuring serum calcium cannot be used as an index of dietary calcium intake. Serum calcium is kept within a very narrow range, regulated by vitamin D, calcitonin, and parathyroid hormone. Excess calcium is stored, so wide variations in calcium intake and absorption cannot be measured by determining serum calcium levels.

Vitamin A and its precursor ß-carotene are also considered critical nutrient modifiers, even though the conversion of ß-carotene to vitamin A is slow and inefficient and the binding and action of vitamin A is a complex process. Different cells have different receptors for subtypes of vitamin A and serum cannot be used to examine the status of these receptors. For instance, giving a patient supplements containing retinoic acid (RA) will increase RA receptor-ß ($RAR_ß$), but not RAR_a, $RXR_ß$ or $RXR_?$. Vitamin A deficiency will result in a decrease in $RAR_ß$ and an increase in TGase I and Keratin K1. The interactions are so complex that simple, straightforward serum level determinations are not possible.

Vitamin E also has been studied as a nutrient modifier of prostate and other cancers. Vitamin E is absorbed into the intestinal tract, enters the mucosal cells, gains access to the bloodstream through the thoracic duct, is transported to the liver, joins the lipoproteins (particularly low-density lipoprotein [LDL]), and is transported into the cell. The bioavailability of vitamin E depends upon how efficiently it is absorbed, how efficiently it is transported in the blood, and how well it binds to serum lipoproteins, which will transport it into the cell. The concentrations of the various binding proteins are critical in determining how much vitamin E the body can access, so measuring the amount of vitamin E in serum will not reveal how much vitamin E is available to the body: only a measurement of the vitamin E-binding protein ratio will do that.

These examples demonstrate the challenge of using the plasma levels of a particular nutrient to determine how well the body will be able to absorb it. Serum calcium is maintained within such a narrow range that serum measurements may not reflect the level of calcium stored in and available to the body; the metabolism of vitamin A is too complex to measure with simple serum tests; and determining the bioavailability of vitamin E means determining the levels of a variety of binding proteins as well. In addition, we do not eat nutrients, we eat food, which is a combination of nutrients. The concentration of one nutrient may be influenced by the concentration of another. For example, the serum concentration of vitamin E is affected by the type of dietary fat consumed, and the absorption of dietary fat is dependent on zinc. A zinc deficiency can have a profound effect on the amount of vitamin E an organism can absorb. These interactions must be considered when plans are made to prevent cancer by adding or eliminating nutrients in the diet.

Perhaps as many as 75 percent of all prostate cancers could be prevented with changes in diet and lifestyle; but scientists must find accurate biomarkers for nutrients and understand the effects of diet on gene expression before they can make useful and effective dietary recommendations. More research is needed to develop better methods of assessing intake, of elucidating the factors that govern bioavailability of dietary components, the significance of interactions among dietary components, the relations of dietary intake to tissue concentrations, and the most appropriate biomarkers of efficacy.

Molecular Pathologies in Exfoliated Cells: Applications in Clinical Prevention

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Exfoliated cells have been used diagnostically for more than a century, and the application of molecular techniques profoundly increases the sensitivity in detecting exfoliated cells. Exfoliation and exfoliated cell pathology applies to any epithelial surface, and research in digestive (specifically colorectal) cancers illustrates the applicability of exfoliation for screening and prevention.

Colon cancer screening currently exists by noninvasive (fecal occult blood testing [FOBT]) and invasive (colonoscopy) means. Each has its drawbacks; FOBT is less expensive but less accurate, and colonoscopy is expensive but detects both cancer and premalignant lesions. A noninvasive, highly efficient tool is being sought. Computed tomography (CT) colonography, or virtual colonoscopy, and swallowed video-capsule technologies are being investigated to meet this goal. Both new methods, however, are invasive and require bowel preparation, which may be a disincentive for some patients. More accurate stool testing is the only noninvasive approach. A number of trials have shown that screening with fecal blood testing over 10 years or more reduces cancer mortality by 12 to 30 percent; however, the majority of those screened in clinical trials that would have died without screening, died with screening.

Exfoliated markers coming from the tumor are continuously released and have a high potential for discrimination, although little is known about exfoliation in this field. In colorectal cancer, there is abundant exfoliation, and the cells are nonapoptotic. Cells can be extracted from stools via density gradient centrifugation, and magnetic beads can be used to separate colonocytes out of the stool. One

problem is that these cells are labile with this technique; detection is reduced as the stool ages. Methods to preserve the cells in a viable state at ambient temperature while in the stool matrix will allow for better recovery of colonic cells for downstream studies of cancer associated markers. An avenue to consider is testing for constituents of colonocytes, such as proteins that can withstand the colonic environment of over 500 taxonomically distinct microflora. Current proteomic methodologies seem well suited for developing colon cancer markers from stool samples.

DNA-based stool testing is biologically intriguing and appealing because there are known alterations in cancer and precancer DNA. DNA, like all exfoliated markers, is shed continuously, yet is stable in stool. In addition, DNA can be amplified before it is measured, which cannot be accomplished with peptide or other markers. Investigators have looked at single markers, particularly *k-ras*, and demonstrated that if the tumor expresses the marker, *k-ras* is usually detected in the stool; however, *k-ras* is expressed in fewer than half of all colon cancers, and the overall sensitivities are disappointingly low. Because of the molecular heterogeneity of colorectal neoplasia, multi-target DNA assays have yielded the highest tumor detection rates. Most prototype assays have included combinations of high-frequency point mutation on p53, APC and K-ras genes, Bat-26 microsatellite instability, and long DNA. Using these assays m reported sensitivities have ranged from about 70-90% for cancer and 50-70% for large adenomas with specificities ranging from 93-100%. Large clinical studies in representative populations are underway to confirm the accuracy of this approach to colorectal neoplasm detection.

There is an opportunity for an expanded value in detecting cancers above the colon using DNA-based stool tests. Approximately 54 percent of all cancers that lead to death are aerodigestive in nature, including lung, colorectal, pancreatic, esophageal, and stomach cancers. All of those excrete DNA into the stool. It may be possible to capitalize on this strategy to screen noninvasively for all of these types of cancers. As such, stool screening may eventuate in a noninvasive and conveniently singular approach for the pan-detection of aerodigestive cancers.

There has been some interest in long DNA—DNA with more than 50,000 nucleotides—as a marker for non-apoptotic shedding. Long DNA is hypothesized to be a hallmark of all malignant exfoliation. Long DNA was present in stools of patients with cancers above the colon, and is not present in normal stools. Using the longest marker size (average log of 2,400 bp amplitude) with 95 percent specificity, about 70 percent of colon and supracolonic cancers can be detected. Cancers in the large airway could be detected more readily than those in the periphery. This is consistent with what has been observed with exfoliated cytology.

In summary, stool DNA screening has the following advantages:

- It comes from the tumor itself instead of a bleeding lesion (i.e., FOBT), and the molecular signature can be specific.
- It has the potential to be highly sensitive in a representative population. Multicenter studies using DNA-based stool screening are being conducted.
- It is noninvasive and user-friendly, requiring no preparation or inconvenience in terms of missing work.

- It specifically tests for the lesion on a molecular level, rather than for semi-related or unrelated abnormalities such as polyps. Most polyps do not become cancerous, and detecting and removing them is a false positive that can lead to a false sense of security.
- Its appeal and value would be enhanced should it result in the detection of aerodigestive cancers.
- Because specimens and not people are tested, it is an efficient way to screen large populations.
- It is cost-effective.

SESSION I: WHAT ARE THE PRACTICAL ISSUES REGARDING THE COLLECTION OF EXFOLIATED CELLS: YIELD, CELL QUALITY, AND DIFFICULTY IN OBTAINING SAMPLES?

Detecting Gene Mutations from Exfoliated Lung Epithelial Cells Obtained in Sputum or From Bronchioalveolar Lavage Fluid

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Lung cancer, mainly associated with smoking tobacco, is the leading cause of cancer-related death in both men and women in the United States. Smoking prevalence among adults has remained unchanged over the past decade and is increasing among teenagers, suggesting this will be a continuing problem. The majority of lung cancers are diagnosed at an advanced, incurable stage, so there is a critical need for improved lung cancer screening.

There are several different histological types of lung cancer. The two most common are adenocarcinoma and squamous cell cancer. The latter forms in the central airways, is visible by bronchoscopy, and is the easiest lesion to detect using exfoliated cells and sputum cytology. It also is most often linked to tobacco smoke. In contrast, adenocarcinoma grows in the periphery of the lung and is much less detectable using exfoliated material. The ratio of adenocarcinoma to squamous cell cancer in White and African-American males and females has risen over the past 25 years, and adenocarcinoma is currently the most common type of lung cancer that is diagnosed. Most of the research looking at lung cancer detection, and specifically at exfoliated materials, often includes only those patients with squamous cell cancer.

Historically two tests have been used to screen for lung cancer: chest X-ray and sputum cytology. The failure of these techniques to effectively detect lung cancer has led to a number of newer tests being developed to diagnose lung cancer in the early stages, including laser-induced fluorescence bronchoscopy, spiral CT scanning and positron-emission tomography (PET) scanning, and the use of early detection markers in sputum, bronchioalveolar lavage (BAL) fluid, and blood. These markers reveal a range of findings such as genetic abnormalities (mutations and loss of heterozygosity), epigenetic changes including methylation, abnormal protein expressions, and proteomic profiles.

The most readily available and noninvasive source of exfoliated material for studies is sputum. Standard cytological techniques usually do not pick up cancerous cells in sputum, which contains few cells from the lining of the respiratory tract. Only 20 percent of diagnosed lung cancers produce positive sputum cytology, and predictive results depend upon tumor location and histologic type. Diagnostic material may be obtained via bronchoscopy; brushings of specific lesions in visible portions of the airway, or bronchoalveolar lavage for peripheral lesions that contain some peripheral airway cells but also contain a high percentage of mononuclear cells.

Molecular changes during lung cancer progression follow the natural history of the disease and are potential targets for molecular screening. In general, early changes can be useful in identifying populations at risk for cancer, but are less specific for diagnosing someone with actual cancer. Changes that occur later in the disease may be more useful in this regard, even for small lesions.

Early studies of abnormal sputum cytology have identified mutations in *k-ras* or *p53* genes that are associated with adenocarcinoma of the lung. One study noted that none of the specific markers were present in all cancers.

Another study, performed in the mid 1990's, identified new markers in blood and BAL fluid obtained during surgery, including methylation of the *p16* promoter and microsatellite alterations, that accurately identified malignancies in more than 80 percent of the specimens analyzed. Since only half the samples of BAL fluids contained tumor-specific abnormalities, tumor location was examined to determine whether this contributed to the poor fluid testing results. Seventy-five percent of the patients in the study had small, peripheral lesions in their lung and 25 percent had tumors in major airways. The *p53* assay was dependent on location and only a small percentage of the peripheral lesions were detected using that test, while the *p16* methylation assay was fairly consistent for both tumor sites. The *p53* assay was also fairly insensitive at the early stage of the disease, but the *p16* assay seemed to be able to detect earlier peripheral cancers. The microsatellite instability was fairly insensitive, *k-ras* had better sensitivity, and methylation seemed to be the most sensitive.

To use these tests for mass screening, the number of tumor cells needed to be increased in the samples or the sensitivity of the assays needed to be increased. A few studies tested techniques to achieve these goals. Mitochondrial DNA was studied since there is a high frequency of mitochondrial DNA mutations in lung cancer. The majority of these somatic mutations were homoplasmic in nature, indicating that the mutant mitochondrial DNA became dominant in tumor cells. Gene sequencing was performed on 15 lung cancers, and at least 40 percent of those cancers had a mutation that could be targeted in a sample. By virtue of their clonal nature and high copy number, mitochondrial mutations may provide a powerful molecular marker for noninvasive detection of cancer.

More research is needed to connect biomarkers with the natural history of the disease. Scientists have found biomarkers in a high percentage of patients at risk for lung cancer, but these findings cannot yet be used to predict long-term outcomes since there is no data on whether biomarkers in high-risk patients translate into cancer at a future date or whether these abnormalities are reversible.

Predicting Breast Cancer Risk by Mammary Epithelium Sampling Techniques

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Phase II chemoprevention studies require serial observation of at-risk epithelium and the ability to repeatedly sample it for biomarker evaluation. One approach to this involves the use of exfoliated ductal epithelial cells obtained from nipple aspiration fluid, or the related technique of ductal lavage (DL). DL involves breast massage, after which a nipple aspirator syringe is placed into a milk duct and 10 to 15 seconds of negative suction produces small droplets of nipple aspiration fluid (NAF). A sample of epithelial cells sufficient for cytologic diagnosis was obtained in 58% of high risk women participating in the original multicenter duct lavage sudy. No topographic map of the number of openings on a breast exists, and the degree of variability between individuals is unknown. Other obstacles for using DL in clinical studies include the inability to secure NAF from all women, the low number of cells recovered, the difficulty in reproducing samples and being able to re-access specific duct sample sites, and the unwillingness of women to repeat the procedure in follow-up studies.

For this test to be of greater benefit to the researcher than fine needle aspiration (FNA), it must be possible to re-test the same duct, reproduce cytologic findings, discover stable biomarkers in the NAF of control subjects, and find the optimal interval between tests, particularly for intervention studies. There is evidence that the epithelial lining is stripped during DL, so there must be adequate time between tests for the lining to regenerate.

A study at Northwestern University used DL to determine which women at high risk of breast cancer were eligible for tamoxifen (TAM) intervention. The NAF was tested for cytologic findings, immunohistic chemical (IHC) markers, the gene methylation profile, and mammographic density. The study included women with small invasive cancers in the early stages of development, but only the opposite breast, not the affected breast, was studied. Seventy-one percent of women yielded DL samples with enough cells for evaluation. Cytologic findings revealed that atypical samples had a much higher cell yield than benign samples. The proportion of atypical cells in these atypical samples was between 20 and 25 percent, and lavage also yielded a mixture of macrophages and non-epithelial inflammatory cells besides mammary epithelial cells.

Complete cytological findings were obtained for 68 women: 18 percent had insufficient samples, 44 percent had benign findings, 34 percent had mild atypia, and 1.5 percent had marked atypia. The median cell count, based on the first quarter of samples, was 4,000 cells and the mean was about 13,602 cells. Of the 171 ducts with complete cytologic data, 40 had less than 1,000 cells. This highlights the need to develop multi-parameter biomarker assessments for a single slide.

Reproducibility is being assessed. So far, 16 of the 68 women have reach the six-month time point for a repeat DL procedure. In all of the women with complete data, at least one duct matched, and in 10 women, more than one duct matched between the first and second procedures. The cytologic findings were only 35 percent concordant and whether this is due to true instability of mild atypia, or is related to the subjectivity of interpretation is uncertain. This might be improved by using biomarkers such as the fluorescence *in situ* hybridization (FISH) probes used by Dr. Bonnie King from Yale University in a study with women who had breast lesions removed surgically. The sensitivity of this test was 30 percent based on cytology and approximately 70 percent when FISH probes were included.

The first such study at Northwestern University randomized 40 women through soy or a placebo intervention with lavage performed at entry and six months later. The study is not optimal, with lower results than current studies, but there was good reproducibility of recannulation of the same duct 6 months later.

How to view histochemistry in DL samples is another issue. Fluorescence labeling is possible on whole cells, not sections, and expensive confocal microscopy needs to be employed to do so. Many of the samples appear to be coming from ducts with some hyperplasia, but this has not been demonstrated.

DL is a useful technique for the serial observation of breast epithelium. When designing a study using DL, at least twice the number of women need to be recruited as the number needed for the study. At least one informative duct cam be recannulated, with sufficient cells for biomarker analysis; but the reproducibility of cytological findings is not significant at this time and the use of biomarkers to increase reproducibility needs to be explored. There are also technical issues with DL, such as the size of the catheter, how to record the duct location, and how to mark the sampled duct so follow-up can be consistent.

There is not a large store of data on cancer detection using DL. DL was performed in the operating room in a study of women undergoing mastectomy for any reason. The DL morphologic findings only detected cancer in 45 percent of the samples taken from breasts with known malignancies. The ability of this test to detect cancer seems poor at this time.

In summary, although DL has the promise of greater reproducibility over time than random sampling techniques, the very preliminary results shown here provide a mixed picture, and more data are required to reach a final conclusion. It appears likely that there is significant attrition in the study population in terms of the goal of replicate biomarker analysis, and recruitment goals may need to be twice the sample size required for interpretation of biomarker data.

SESSION II: WHAT HAVE WE LEARNED FROM STUDIES WITH EXFOLIATED CELLS?

Biomarkers for Chemoprevention Studies: What Can Nipple Fluids Tell Us?

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Compared to research on exfoliated cells in the colon and lung, there is minimal literature available on exfoliated breast cancer cells in nipple fluid. This seems to be an area that could prove beneficial because of the wealth of biochemical elements (nucleic acids, proteins, lipids, cholesterol, steroid hormones, exogenous carcinogens) and epithelial cells found in nipple fluid. Some research has been completed on the use of breast epithelial cells as risk markers when evaluating women for possible chemoprevention, although these cells may also be useful as markers of drug effects and surrogate endpoint biomarkers.

Results from clinical studies of DL cytology have identified the following areas of concern:

- Diagnosis may not be reproducible between cytologists. The results from a single cytologist have good reproducibility, but tests done at another center may not reproduce the original findings.
- Diagnoses may not be reproducible over time, and cells may be affected by the menstrual cycle.
- There are concerns about whether or not cell production might be decreased after successful chemical interventions. For example, women who take tamoxifen are less likely to produce nipple fluids, thus it may reduce cell counts.

Chromosomal instability has been studied using nipple fluid, and these studies have demonstrated that virtually all breast cancers have gains in chromosomes 1, 2, 8, 11, 17, and 20, and some losses on 17p. Individual genes that have been identified as potential risk markers are *c-MYC*, *HER2*, *cyclin1*, and *p53*. Probes using fluorescent *in situ* hybridization, have been developed for cells obtained by fine nipple aspiration (FNA) that have approximately 90-percent sensitivity between biopsy-proven cancer and FNA cytology. Studies are currently being conducted using DL specimens from women who are at high risk for developing cancer, although the same levels of chromosomal instabilities have not been identified in tumors from these women.

A small feasibility study, involving 25 healthy volunteers, 80 percent of whom were pre-menopausal, was conducted to identify protein, DNA, and mutagens in nipple fluid. The study addressed whether or not DNA could be amplified from nipple fluid. Mutagenesis assays were preformed using the Salmonella (Ames) assay. DNA amplification of several microsatellite regions was carried out using polymerase chain reaction. The ability to amplify DNA ranged from 34% to 96%, depending on length of the microsatellite region examined. The average protein concentration was 71µg/mL. Two-dimensional protein electrophoresis was successfully performed on samples from two subjects.

Another study is focusing on core needle biopsies to obtain breast tissue from patients enrolled in chemoprevention studies. Women seem to tolerate this intervention, and paired pre- and post-therapy samples that contained epithelial cells have been obtained from 88 percent of the specimens. The samples are being frozen and stored until the investigators choose the biomarkers they want to investigate.

Evaluating exfoliated cells may be a good way to look for biomarkers of risk, and this technique can be used in small exploratory studies. It is unclear if this is the best approach for chemoprevention trials. Some small pilot studies are underway that may help answer some of the questions still remaining about the use of exfoliated cells in chemoprevention. These questions include the variability of biomarkers between pre- and post-sampling, how to evaluate the changes that occur during the trial period, and whether interventions change biomarkers. In summary, cytology may currently be the most clinically relevant biomarker in nipple fluid, but genetic and molecular markers may ultimately complement or prove more informative than cytology and the choice of breast sampling technique for epithelial cells depends on the study question and local expertise.

Dynamics of Carotenoid Turnover in Exfoliated Colonic Epithelial Cells

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The gastrointestinal (GI) track undergoes rapid renewal, during the course of which millions of cells are shed into the fecal stream. The entire mucosa is renewed every five days, which creates a very rich source of viable exfoliated cells in stool that makes sampling easy and efficient. A significant number of these cells can be recovered in a viable state from a small stool sample. The cells isolated from stool have unique characteristics that tell a great deal about the entire organism and reflect functioning in organ systems outside the GI tract. Stool samples also maintain 85 percent their viability for four to five days after collection. They do not need refrigeration, and rather simple laboratory procedures and equipment can separate relevant cells from the sample.

One process for obtaining exfoliated cells from stool is Somatic Cell Sampling and Recovery (SCSR), which is a true sampling of the entire colonic mucosa. Cells in excess of 10 million/gm (up to 30 million/gm) are obtained. Samples from people on a high fiber diets produce much higher cell yields, and the exfoliation rates are higher as well. Cell size ranges from 2 to 8µM and there are two distinct populations (cells between 2 and 5 µM and cells between 5 and 8 µM), each anatomically representative of the colon. Fifty percent of the cells express blood group antigens and come only from the proximal half of the colon. The exfoliated cells from the terminal half of the colon are negative for blood group antigens. Cells from the small intestine, stomach, duodenum, and esophagus have not been found in the colon. These cells exclusively have colon biomarkers and thus essentially come from colonic mucosa.

There is a concentrated range of nutrients in the exfoliated cells, including significant amounts of a-tocopherol, ?-tocopherol, and retinal lutein. Lycopene, a-carotene and ß-carotene have not been found in the samples. Cells exfoliated from the colon seem to sequester more ?-tocopherol than a-tocopherol. A study of markers to measure ß-carotene involving subjects that consumed kale, spinach, or tomatoes over a period of five days indicated that levels reach their peak between day 5 and day 7. There also is a lag in the appearance of the marker lycopene between days 5 and 7, and lutein uptake involves a lag of 5 to 6 days.

A time course study in India revealed that subjects on a high ß-carotene diet showed low concentrations of ß-carotene, while subjects consuming a low ß-carotene diet for 7 days, followed by high ß-carotene intake, reached peak levels of ß-carotene at 11 or 12 days. These results have led researchers to suggest that the mucosa exfoliates in very specific ways.

SCSR provides viable exfoliated epithelial cells that reflect the subjects' immediate past nutrient intake, metabolic activity, mucosal turnover rates, and gene expression. For example, mucosa cells rapidly divide in the lower third of the colon, and as they migrate upward they lose their proliferative capacity and finally are exfoliated from the surface. This division and migration process takes between 5 and 7 days. What might be happening is that the nutrients initially are absorbed and go into systemic

circulation as these cells migrate upwards, after which they exfoliate and are excreted in stool. It is possible that this concept can be used to determine the turnover rates of colonic mucosa.

Changes in Gene Expression in Exfoliated Cells: The Role of Bioactive Food Components

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Early detection of colon cancer can result in a high cure rate. Therefore, an accurate screening method is imperative. Consistent with this goal, adoption of noninvasive methodology designed to reduce anxiety over colorectal cancer screening and improve overall acceptance of the screening process would be highly desirable. Unfortunately, current noninvasive methodologies lack sensitivity and will not detect alterations in gene expression. For example, markers for colorectal tumors have been detected in the stool of patients following fecal DNA analysis and detection of oncogene mutations using polymerase chain reaction. However, disadvantages on this noninvasive methodology are: (1) a lack of sensitivity because detectable mutations may be confined to a very small number of tumor cells; and (2) the inability to detect alterations in gene expression. Because activation of proto-oncogenes and inactivation of tumor suppressor genes can occur by epigenetic mechanisms (i.e., there is no change in DNA base sequence, rather a change in gene expression), in addition to genetic mechanisms (i.e., expression of genes altered through point mutation, truncation. Or translocation), mRNA compared to DNA-based methodology has broader application and diagnostic value in monitoring the detection of colon cancer. Therefore, we have developed noninvasive methodology utilizing feces containing exfoliated colonocytes to quantify colonic mRNAs. Although RNA generally is less suitable than DNA because it is readily degraded, our data demonstrate that intact fecal eukaryotic mRNA can be isolated because of the presence of viable exfoliated colonocytes in the fecal stream.

Using noninvasive techniques, animal models of colon cancer were monitored to observe the effect of nutrition on colon cancer susceptibility. Although the number of apoptotic cells that can be detected in a crypt in both animals and humans is small, a large number of exfoliated cells are continuously being shed and pass into the colon. Looking at biomarkers in colon cancer tissue, especially in humans, is labor-intensive. It is easier to look at the genomes expressed in exfoliated cells to monitor the state and progress of colon carcinoma.

This study evaluated the effect of nutritional components, especially omega-3 fatty acids, on the prevention of colon cancer by observing how these components affect the genes in exfoliated colon cancer cells. The genomes of such cells also were studied for elements that (1) could predict an animal's risk of developing colon cancer, (2) could be used as a diagnostic classification tool, and (3) could be used to study genetic mutations or long DNA. Theoretically, RNA can be pulled from the feces, and protein might also be pulled, as long as the cell is intact and has not been subject to degradation by the harsh environment of the lumen.

This study hypothesized that levels of the PKC isoenzymes beta-2 and zeta could predict an animal's risk of developing colon neoplasia, and that these levels were affected by both carcinogens and

diet. PKC-beta 2 and zeta have contrasting functions in the colonocyte: beta-2 encourages proliferation, and zeta blocks tumor formation.

Forty-eight rats were divided into groups and fed two lipid solutions (corn oil which is chemopromotive and fish oil which is chemopreventive) and two combinations of fiber, plus or minus carcinogens (azoxymethane, a strong alkylating agent). The study results validated the hypotheses. The level of PKC-zeta was significantly lower, and the level of PKC-beta-2 was significantly higher in tumor-bearing animals than in animals that were tumor-free. Both of these isoenzymes may have roles in monitoring the neoplastic process. Animals that received the diet supplemented with omega-3 fatty acids had lower PKC-beta-2 levels and fewer tumors.

In the next study, mRNA was isolated from human stool samples to assemble a panel of biomarkers and assess the impact of nutrition on the selected biomarkers. Twenty-three human subjects were studied. Seven were healthy, six had colonic inflammation, and 10 had adenomas. Colonoscopies were performed and fecal samples were collected at that time. Total RNA was extracted from the mucosa using a molecular sieving approach. RNA samples were tested for markers elevated in the mRNA of colonic tumors, including cyclin D1, beta-catenin, c-myc, PKC-zeta, PKC-beta 1, PKC-beta-2, PKC1, p53, LDH, survivin, and cyclooxygenase-2 (COX-2). Cyclin D1 levels reached significance in the adenoma group. The level of COX-2 was not higher in patients with adenomas, but was significantly increased in the group with chronic inflammation, and might be a useful marker for the presence of ulcerative colitis

Additional studies are planned to analyze exfoliated cells from fecal samples for predictive biomarkers for colon cancers. Questions that also need to be addressed include how fecal mRNA expression compares to mucosal profiles, whether or not a single gene can predict the risk of developing cancer or if combinations of gene sets must be used, how to separate treatment groups on the basis of gene expression profiles, and whether the mRNA in stool comes from the exfoliated cells or from some other source. Another investigation that could potentially reveal crucial information is following people with inflammatory bowel disease to see how and when their lesions progress into cancer. Since colon cancer is primarily a disease of aging, monitoring gene expression changes over time will allow scientists to evaluate the changes aging produces in an organism's genetic make-up.

Cytologic and Methylation Changes in Exfoliated Bronchial Cells Predict Lung Cancer. But Why?

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Abnormal cytology can predict lung cancer risk years into the future, as does the methylation of tumor suppressor genes. It is important to determine whether these are larger field effects or innocent bystanders that seem to correlate with lung cancer. A decade ago, the University of Colorado Lung Cancer Specialized Program of Research Excellence (SPORE) began collecting respiratory tissues from large numbers of people with moderately severe chronic obstructive pulmonary disease (COPD), at the beginning from sputum and then through bronchoscopy. This project became a cohort study, which

now includes 3,000 subjects with COPD. A decision was made in the past year to stop recruiting people with COPD and add 700 to 900 people without COPD to the protocol.

At baseline, participants had sputum collected and also provided information on their risk factors for lung cancer, including nutritional information. Sputum samples are taken and the risk assessment is performed annually. During the first 6 to 8 years after enrollment, abnormal cytology can predict lung cancer risk with hazard ratios of approximately 3.0. The Colorado researchers are now collaborating with the Johns Hopkins Lung Cancer SPORE, and sputum samples from both venues are sent to New Mexico, both from subjects who later developed lung cancer and from those who did not. The first 33 nested case control pairs have been analyzed, and two genes that seem to be implicated in lung cancer have been found: *p16* and *Mgmt* methylation in the sputum. Results will soon be published. Over the summer, the researchers will review an additional 50 case control pairs for these two genes and five others.

The investigators appear to be viewing exfoliated cells not for cancer, but for large field effects that indicate high risk. They have found general molecular risk profiles that indicate peripheral as well as central airway lesions. Genetic hyper-methylation predicts lung cancers well, although the specificity is poor in high-risk subjects (perhaps because more than half of the current subjects have hyper-methylation suppressor genes). Methylated genes appear not to be specific for central airway lesions, but there are morphologic changes in both the exfoliated cells and methylated genes that predict lung cancer. Interestingly, when the team found methylated genes within dysplastic tissues, they rarely found them in the patient's sputum. This suggests that it is not just dysplastic tissues that are shedding methylated cells, and that hyper-methylated tumor suppressor genes are crossing a wider range. What initially looked like discrepancies are pointing to field effect observations. This is supported by the fact that 28 of the first 33 case control pairs were methylated and 20 of the 33 controls were also methylated. In addition, it is not unusual for a subject to be methylated one year and not methylated the next. Atypical cells are almost always associated with current smoking, while fruit and vegetable intake is associated with lower levels of atypia.

The increasing number of case-control pairs will enable a number of issues to be studied in more detail, including the connection between histology and tumor location, and whether or not tumor methylation and methylation of the sputum are related. The prediction is that there will be no relationship, that they are both representative of some other common cause.

Analyzing atypical cells in sputum may be the most appropriate way to identify central airway lesions, whereas CT scans may be more useful in tracking down peripheral malignancies. Another method of predicting lung cancer may be examination of the stool If single genes or a panel of genes are found that indicate a high risk for lung cancer among smokers and former smokers, they could be identified in stool as well as in sputum samples.

SESSION III: ARE DIETARY-INDUCED CHANGES IN BIOMARKERS THAT ARE MEASURED IN EXFOLIATED CELLS INDICATIVE OF CHANGES IN TARGET

TISSUES, OR ARE THEY REPRESENTATIVE OF GLOBAL CHANGES WITHIN THE BODY?

Tissue Specificity: Comparison of Lycopene Accumulation and DNA Damage in Exfoliated Human Lung Epithelial Cells and Lymphocytes

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A clinical trial was performed that compared carotenoid levels and DNA damage (single strand breaks [SSBs]) in leukocytes, with carotenoid levels and SSBs in the macrophages and epithelial cells of healthy human lung tissue, respectively, both before and after the research subjects took antioxidant supplements and were exposed to ozone. Thirty-one healthy, non-smoking subjects between the ages of 18 and 35 years completed the trial, and we examined samples and data on 23 of these subjects. They recorded their food intake daily, and consumed a low fruit and vegetable diet for the entire three weeks of the study, so that everyone shared the same depleted level of vitamin C and carotenoids. After one week of dietary restriction, all participants exercised for two hours (15 minutes on and off) in a chamber filled with ambient air. After a bronchoscopy and BAL, all subjects were randomized to either the supplement or placebo group. For the next two weeks, the supplement group received one can of V-8 juice and vitamins C and E daily, and the placebo group received orange soda and placebo pills every day. After the dietary intervention, all subjects were exposed to ozone while they performed the same set of exercises, and a second bronchoscopy and BAL were performed. Macrophages were obtained by sterile saline lavage during the bronchoscopy, and brush biopsies were done to obtain lung epithelial cells.

Using high performance liquid chromatography (HPLC), the study compared plasma carotenoids with lung macrophage carotenoids. DNA damage was measured in leukocytes and lung epithelial cells with the Comet assay. During a Comet assay, cells are placed in agarose gel on a microscope slide and soaked in a lysing solution, after which the DNA is allowed to unwind in an alkaline buffer. Single-cell gel electrophoresis is performed; the slide is rinsed in Tris, neutralized, and stained with ethidium bromide. Comet-like images on the slide represent damaged DNA, and the extent of DNA damage can be analyzed by measuring the length of the comets "tails." The study scored 100 cells per sample, and measured the average comet area and the average comet tail length.

Carotenoids in samples of the vegetable juice were measured, and trans-lycopene was dominant, although some beta-and alpha-carotene were seen as well. Carotenoid levels did not change significantly in either the plasma or lung macrophages in the placebo group during the study (there was a slight decrease). The subjects who received supplements achieved increases in all plasma carotenoids except beta-cryptoxanthin, which was not present in the vegetable juice. There was a significant increase in alpha-carotene in the supplement group's lung macrophages, and less significant increases in *cis*-lycopene and *trans*-lycopene. All other carotenoids decreased in lung macrophages of supplemented subjects. The correlations between levels of carotenoids in plasma and lung macrophages was weak, and the correlation between carotenoids in the diet and lung macrophage

carotenoids was weaker. After the dietary intervention, the correlations between dietary carotenoids and plasma carotenoids were much higher than those reported in the literature. This may be due to the subjects maintaining a consistent diet. The supplemented group did not sustain DNA damage in either their leukocytes or their lung epithelial cells, but the placebo group had a significant increase in comet area and comet length in their lung epithelial cells after exposure to ozone. The DNA in the placebo group's leukocytes remained stable.

In conclusion, although lung macrophage and plasma carotenoids were manipulated by dietary intervention, plasma carotenoids were not correlated with lung macrophage carotenoid concentrations. Additionally, changes in DNA damage in lung epithelial cells were not reflected in DNA damage measured in blood lymphocytes following antioxidant supplementation and ozone exposure.

Relationship Between DNA Methylation in Exfoliated Cells and Target Tissues

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Recent studies have indicated that global DNA hypomethylation, an epigenetic event, plays a major role in tumor formation, possibly by promoting chromosomal instability. Epigenetic events are susceptible to change and are often vectors that allow environmental factors, including diet, to modify cancer risk and tumor behavior. Most of the previous studies on DNA methylation were conducted using a radiolabeled methyl incorporation (RMI) assay. Recent and ongoing studies employ an immunohistochemical assay that uses monoclonal antibodies against 5-methylclytosine (5-mc). In methylation, cytosine is converted into 5-mc, and the immunohistochemical assay evaluates the degree of global DNA methylation by visualization of the antigen-antibody complex (a brown reaction product) in cells of interest. Although immunohistochemistry is not a linear technique, a reproducible semiquntitative index of immunohistochemical reactions, incorporating both the intensity of staining of individual cells and the proportion of cells staining at different intensities, can be developed by experienced researchers. The ability to assess methylation status in specific types of cells involved in the process of carcinogenesis is an important advantage of the immunohistochemical technique. In the RMI assay, radiolabeled methyl groups incorporate into the DNA when it contains cytosine rather than 5-mc, so the RMI evaluates the extent of methylation by measuring the inverse relationship between methylation and how much radiolabeled material has been incorporated. Since DNA must be extracted to perform RMI assays, the test cannot be used to evaluate methylation status in specific types of cells. Global methylation levels go down in cancerous tissues, and previous studies using RMI suggested that altered global DNA methylation in target tissues is a nutrient-related biomarker that might be useful in detecting the presence of precancerous or cancerous lesions. Most published studies have been performed on the lung and the cervix.

It is likely that alterations in global DNA methylation in target tissues may be a better indicator of cancer risk than methylation levels in the blood or other areas of the body away from the at-risk tissue. Unfortunately, since obtaining target tissue samples is invasive, methylation indices may not be

suitable for cancer prevention or early detection efforts unless tissues such as peripheral leukocytes, which can be easily obtained, are used for evaluation. It is not clear whether or not methylation levels in peripheral tissues reflect changes in target tissues. The relationship of global DNA methylation to target tissue methylation was recently investigated, using the RMI assay, by obtaining several different tissue samples from each subject in a study of patients diagnosed with non-small cell lung cancer. Peripheral leukocytes were isolated from a blood sample; and buccal mucosal cells and malignant and non-malignant tissues were also collected. RMI assays showed a significant correlation of DNA methylation status only between buccal mucosa cells and malignant tissues of the lung.

Because 5-mc assays allow different parts of a specimen to be separately evaluated, they are useful in understanding the role of global DNA methylation in the process of carcinogenesis. The 5-mc assay also can evaluate global DNA methylation in exfoliated cells from different sites in the body, including buccal mucosal cells, urinary tract cells, broncho-alveolar lavage cells, and cervical cells.

Little research has been conducted to demonstrate what the differences are in staining levels by diagnoses and whether staining intensity is lower in cells from the urinary tract, buccal mucosa, and cervix. A study has been performed on cervical intraepithelial neoplasia (CIN) using the 5-mc process. The intermediate target was exfoliated cells, and the ultimate target was cells obtained during a cervical biopsy. Cells collected with a cervical brush include different types of cells such as lymphocytes, neutrophils, squamous, metaplastic and glandular cervical cells, and the 5-mc assay is able to assess varying degrees of methylation in these cells.

Because squamous cell cancer is the most common neoplastic lesion of the cervix, the study limited its methylation analysis to metaplastic or squamous cells. The level of 5-mc in exfoliated cervical cells depended on the severity of the dysplasia present, with low levels of dysplasia producing low levels of 5-mc positive cells. DNA methylation patterns were examined in biopsy specimens of normal cervical epithelium and tissues that contained CIN 1, 2, and 3. The cells of both normal tissue and tissue displaying CIN 1 lesions were poorly methylated: the number of methylated cells was much higher in CIN 2 and 3.

Researchers also studied whether or not there was an association between the percentages of cells positive for 5-mc in biopsy specimens and exfoliated cervical cells. Exfoliated cells were compared to a combination of cells from CIN 1, 2, and 3 lesions, and a significant positive correlation was noted. When the analysis was limited to exfoliated cells and CIN 3, the correlation was even stronger. These preliminary results suggest the possibility of using the status of global DNA methylation in exfoliated cells of the cervix as a biomarker for cervical dysplasia. Before 5-mc assays can be used in large, population-based studies, the techniques for capturing, enriching, and preserving exfoliated cells must be improved. Enough cells may be obtainable for accurate study from the cervix, but bronchial lavage might not produce the required amount. If protocols other than the 5-mc assay are used to evaluate methylation status, techniques need to be developed to separate cells of interest from other cells, since an evaluation of methylation in a mixture of cells is likely to yield results with low sensitivity and specificity. To apply these techniques for large population-based studies of biomarkers, techniques need to be improved for capturing, enriching, and preserving exfoliated cervical cells.

Effects of Ingested Arsenic on DNA and Chromosome in Human Exfoliated Epithelia

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Chronic exposure to arsenic has been associated with skin, lung, and bladder cancers, as well as noncancer effects such as cardiovascular diseases. There is great uncertainty about the health effects at low doses. Most animal studies are negative for carcinogenesis from arsenic exposure. No appropriate animal models are available to investigate the mechanisms of arsenic carcinogenesis. Research is needed to understand mechanisms for arsenic carcinogenesis in humans and also to develop sensitive biomarkers for health risk assessment at low dose. Methylation is important in the metabolism of arsenic in humans; methylation was thought to be a detoxification element. Recent studies suggest that methylation may enhance the toxicity of arsenic.

The ground water in an arsenicism endemic site in Ba Men was contaminated with arsenic due to the natural geological formation. In this area, skin hyperkeratosis, which is a hallmark of the skin lesion from chronic arsenic exposure, was commonly found among the residents here. In addition to skin cancers, neurological effects were also found from high exposure to arsenic. Studies also show cardiovascular effects, including abnormal ECG. The Inner Mongolian population is of interest because the Environmental Protection Agency needs human data on health effects of arsenic exposure via drinking water. In this population, the main route of arsenic exposure is from drinking water. Eighty percent of the residents have their own wells. This makes exposure assessment of individuals possible. They also have good nutritional status, so malnutrition is not a confounding factor in the study.

The Mongolian study investigated the health effects from chronic arsenic exposure, and sought to identify biomarkers useful for assessing arsenic exposure and health effects, especially at low doses. Using exfoliated cells, this study analyzed chromosome damage and DNA fragmentation. Buccal cells, airway epithelial cells in sputum, and urothelial cells (mainly bladder cells) were collected and analyzed for the frequency of micronuclei to assess chromosome damage. Buccal cells were assayed for DNA fragmentation using DNA laddering by gel electrophoresis, and terminal deoxyribonucleotide transferase-mediated dUTP-X Nick End-Labeling (TUNEL) method. Results of this pilot study indicated that chromosome and DNA damage were associated with chronic exposure to high levels of arsenic.

A larger study was conducted using low, medium, and high arsenic dosage levels for comparison of participants. Buccal cell samples were collected to investigate the effects of arsenic on DNA and chromosome damage. About half of the participants were smokers, and half were female. Ages ranged from 10 to 60. Even at a young age, hyperkeratosis was present, although the effects of arsenic exposure were highest among older participants. DNA fragmentation appears to have a threshold effect and only showed effects in the high exposure group. Whereas elevated frequency of micronuclei was shown starting in the medium exposure group. This study showed that chronic exposure to arsenic is associated with increased DNA and chromosome damage, which may increase the risk of cancer and other chronic health

effects. Detecting micronuclei and DNA fragmentation in exfoliated epithelia may serve as a useful biomarker for assessing health effects of arsenic exposure via ingestion.

A biomarker study in southern China investigated lung cancer and indoor air pollution. Women in this area have high rates of lung cancer mortality, even among nonsmokers. Residents in the study area burn coal in open pits in their homes without chimneys and are exposed to carcinogens produced by burning coal. The most common type of adenocarcinoma among this group was bronchioalveolar carcinoma. Biomarkers were identified for assessing dosimetry, urinary metabolites, genetic susceptibility and mutation in target genes (i.e., *p53* and *k-ras* in sputum cells). Studies in buccal cells showed that *GSTM1*-null can increase cancer risk, and *GSTT1* is not associated with lung cancer risk. This study showed that it is possible to use the exfoliated cells to investigate genetic susceptibility and genotoxicity for assessing cancer risk in humans.

FUTURE DIRECTIONS

The use of exfoliated cells for prevention, detection, and treatment of cancer by nutritional modulation is in its infancy, and coordination of efforts in the research community are not at a level to assure beneficial outcomes from ongoing research at this time. In addition, research is beginning to offer a better understanding of how nutrients and bioactive compounds are metabolized. There still is much work to be performed in this area, which will be key to understanding how nutrition is related to cancer prevention. Selected questions remaining to be answered include:

- Are DNA adducts in exfoliated cells important, and how are they modulated by dietary constituents?
- What is known about biomarkers of exposure that validate epidemiologic nutritional intake, and what is the best way to validate these biomarkers?
- Are there biomarkers for early detection of carcinogenesis that can be identified with the same certainty as blood pressure is identified as a marker for heart disease?
- Are there markers that might be endpoints of nutrition modulation or chemoprevention, and can these be validated?

There are opportunities and challenges in all of these areas.

Where do we go from here?

- In order to develop coordination among researchers studying nutrition and exfoliated cells, it will be important for each investigator to demonstrate that what they are studying in exfoliated cells is relevant to what is actually occurring in the tissue that is developing cancer. This is of critical importance to be able to answer the many questions remaining about the application of this research to cancer prevention.

- There is a need to have an accurate quantitation of tissue biomarkers of nutritional dose that are separate from plasma levels of the biomarker.
- There will be an announcement soon asking researchers with NIH or NCI funding to send biological samples to NIH or NCI for inclusion in gene expression microarrays. If researchers are working on animal studies, they may send tissue samples (e.g., colon, prostate, breast, or lung) to NIH or NCI, and these will be analyzed, with results placed on the Web. See http://www3.cancer.gov/prevention/funding.html for more information.
- A mechanism could be considered for collecting exfoliated cell samples from ongoing clinical prevention trials.
- Consideration should be given to developing a scoreable index that demonstrates the risk of cancer based on proteomic data using micro- or nano-technology.
- There appears to be enough research on GI and lung cancer to begin concentrated research on exfoliated cells in those areas; research in breast cancer and exfoliated cells is not as advanced and may not be as productive as GI and lung research.
- Development of a Web site specifically for NCI-funded intervention trials on nutrition, stratified by nutrient should be considered. Information on alterations in biomarkers could be added to the site as data becomes available.
- Individual variability in response to nutrients, given the complex phenotypes present in the human population, represents a considerable challenge in nutrition research. There may be a benefit in looking at this area before beginning large nutrition trials.
- Methylation of genes in relation to exfoliated cells may be an area of potential research that can have applications to cancer prevention as well as cancer treatment modalities.
- The NCI has a large collaborative agreement (U54) with institutions focused on examining the impact of nutrients on genetic pathways involved with cancer. A re-release of this funding mechanism will occur shortly, and applications for the 5-year grants will be due in 6 months. This may be an opportunity for people involved in exfoliated cells to form a collaborative effort and to bid for one of the grants.
- The minimum length of time to create a viable bioassay for exfoliated cells would probably be very short, and this could help move the field ahead dramatically. These tests should focus on endpoint analysis.