Cancer Biomarkers 3 (2007) 61–63
IOS Press

Session VI: Colon

Colon cancer: Recent developments and opportunities for improved prevention, detection, and treatment

Randall W. Burt
University of Utah School of Medicine, Huntsman Cancer Institute, Salt Lake City, UT 84112, USA

Remarkable advances have recently been made in the basic and clinical science of colon cancer, including those addressing inheritance, epidemiology and risk factors, genetic pathogenesis, screening and prevention, diagnosis, and therapy. Nonetheless, incidence and mortality have changed little; this disease remains at epidemic levels in the US and most western countries. Scientific advances and emerging technologies must be translated into more effective cancer prevention, diagnosis, and treatment strategies.

Epidemiologic investigation has demonstrated a direct association of high fat and red meat diets, pelvic radiation, and sedentary lifestyle with colon cancer occurrence. Inverse associations in decreasing order of magnitude include nonsteroidal antiinflammatory drug (NSAID) use, regular exercise, and diets high in vegetables, fruit, and fiber. Prospective dietary interventional studies, however, have shown no decrease in adenoma formation, and NSAID use for decreasing risk is not yet recommended. Perhaps the most common and well-characterized risk factor for colon cancer is family history. Screening strategies now include family history to determine the age and frequency of screening; genetic diagnosis is available for the inherited syndromes of this malignancy.

Colon cancer screening with fecal occult blood testing and sigmoidoscopy decreases colon cancer mortality, but colonoscopy, by inference, is considered the best screening tool. Its cost, complexity, risk, acceptability, and availability, however, present substantial issues. Genetic and imaging approaches, including virtual colonoscopy (CT colography) and PET imaging, are modalities of considerable interest that may maintain the accuracy of colonoscopy while making screening more acceptable.

New surgical, chemotherapeutic, and radiation therapies have recently improved both cure and survival rates for colon cancer patients, but far better therapies are needed. It is hoped that advances in understanding the genetics of colon cancer will lead to precise risk determination, genetically directed screening and diagnosis, novel imaging diagnostics, and genetically targeted molecular therapies.

Understanding the molecular pathogenesis of colon cancer began with the discovery of the tumor suppressor adenomatous polyposis coli (APC) gene as it mutated in the rare inherited syndrome of familial adenomatous polyposis (FAP). Soon after that discovery, it became apparent that over 80% of all colon cancers began with inactivation of this same gene, albeit somatic inactivation in sporadic cases. Additional study found that polyp-to-malignancy progression involved mutation of multiple additional genes, including K-*ras*, p53 and others. Another rare syndrome, hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, arose from mutations of mismatch repair (MMR) genes, including *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Other mutations followed inactivation of MMR genes, including *TGF*β, *BAX*, *BAT26* and other gene products. Similar to APC, 15% of sporadic colon cancers included somatic inactivation of MMR genes.

Genetic testing for FAP and HNPCC is now part of standard clinical practice; cancer screening in persons and families with these conditions is based on the genetic result. As up to one-third of colon cancer cases occur in the setting of familial risk, it is expected that additional susceptibility genes will soon be identified.

Genetic diagnoses based on finding mutations in the genes involved in colon cancer pathogenesis in the cellular debris of stool is already a reality, although much improvement is needed for practical application. It is anticipated that genomic and proteomic approaches will lead to precise serum diagnostics and that these same methodologies will be used to characterize colon

ISSN 1574-0153/07/$17.00

tumors for more individualized therapies. Additionally, genetic-based imaging diagnostics and genetic-based targeted therapies are much needed areas of investigation.

Development of a colorectal cancer risk assessment tool

Ruth M. Pfeiffer[a], Andrew N. Freedman[b], Mitchell Gail[a], Rachel Ballard-Barbash[b] and Marty Slattery[c]
[a]*National Cancer Institute, Division of Cancer Epidemiology and Genetics, Biostatistics Branch, Bethesda, MD 20892, USA*
[b]*National Cancer Institute, Division of Cancer Control and Population Sciences, Rockville, MD 20852, USA*
[c]*University of Utah, Salt Lake City, UT 84132, USA*

Several modifiable risk factors have been consistently identified for colorectal cancer. To date, models to estimate absolute colorectal cancer risk only exist for special populations. We developed a statistical model to estimate the probability of first incidence of proximal, distal, or rectal cancer in white men and women ages 50 and older, over a defined period of time. Using logistic regression, we estimated relative risks separately for proximal, distal, and rectal cancer for a number of previously identified risk and protective factors. We used data on white men and women ages 50 and older from two large population-based case-control studies of colon and rectal cancer, conducted in Utah and Northern California Kaiser Permanente between 1991 and 2001. Age-specific incidence rates from the Surveillance Epidemiology and End Results (SEER) study and attributable risks were used to compute baseline age-specific hazard rates. National mortality rates were used to estimate the hazard for competing causes of death. Risk and protective factors for colorectal cancer in the model include sigmoidoscopy in the last 10 years, history of polyps, family history of colorectal cancer, vigorous exercise, regular aspirin/NSAID use, smoking, body mass index (BMI), hormone replacement therapy use and vegetable intake. Relative risk estimates and risk factors differed among proximal, distal and rectal cancer sites. We also developed a short questionnaire (five to eight minutes) to estimate risk factors for the model and validated it by cognitive testing. We plan to extend the colorectal cancer model to blacks and Hispanics using SEER baseline hazard rates, and validate it in several large cohort studies.

FDG-PET and FDG-PET/CT virtual colonoscopy as a potential surveillance tool in populations at high-risk for colon cancer

John M. Hoffman
University of Utah School of Medicine, Huntsman Cancer Institute, 84112, USA

FDG-PET, as well as the more currently used FDG-PET/CT imaging, is now widely used for staging, restaging, and therapeutic monitoring of patients with various malignancies, including colorectal cancer. Of interest over many years has been finding incidental focal colonic and small bowel uptake of FDG, felt to be of no clinical significance. Evaluated in more detail, however, focal nodular or nodular multifocal FDG-PET uptake in the colon was associated with a pathologic process including malignancy in a majority of instances. Further studies confirmed that the presence of incidental focal colonic FDG uptake on a PET/CT scan justified a colonoscopy to detect (pre-) malignant lesions. Virtual colonoscopy continues to be studied as a less invasive screening tool than colonoscopy. The study and evaluation of virtual colonoscopy for surveillance of both routine and high-risk populations continues to evolve. Combining anatomic detail from virtual colonoscopy with metabolic information obtained with FDG may improve the accuracy of either test alone. In certain populations at risk for colon cancer, such as patients with FAP and attenuated FAP, use of FDG-PET/CT virtual colonoscopy may allow for less invasive surveillance of patients and assessment of overall polyp burden as well as detection of advanced lesions that may have developed in the stomach and small bowel in addition to colon. It is doubtful that the technique would replace standard colonoscopy; however, it may prove useful in an adjunct role to assist with surveillance in these complicated patient groups.

Spectral karyotyping detection of colon precancer

Hemant K. Roy
Evanston-Northwestern Healthcare, Evanston, IL 60201, USA

Colorectal cancer remains the second leading cause of cancer deaths despite the ability of colonoscopy to prevent 75–90% of cancers from developing. However, screening the entire average-risk population ($>$ 70 million Americans over age 50) is impractical given

the expense, lack of sufficient endoscopic capacity, and complication rates. Therefore, limiting colonoscopy to those patients who harbor polyps/carcinoma is essential for efficacious and cost-effective colorectal cancer screening. Many risk-stratification techniques currently exploit the field effect, the proposition that the genetic and environmental milieu that leads to tumorigenesis in one area of the colon should be detectable, at least in some form, throughout the colon. Current markers of the field effect (e.g., distal adenoma on flexible sigmoidoscopy) lack sensitivity for proximal lesions.

Our multidisciplinary colorectal cancer prevention group has developed two novel light-scattering technologies: four dimensional elastic scattered light-scattering fingerprinting (4D-ELF) and low-coherence enhanced backscattering spectroscopy (LEBS). This allows a heretofore impossible quantitative assessment of the nanoscale architecture of cells in order to provide a practical marker for genetic/epigenetic changes of the field effect. Initial studies in two experimental models indicated that altered light-scattering signatures from the histologically normal mucosa preceded the earliest conventional markers of colon carcinogenesis. Preliminary clinical studies on $\sim$200 patients demonstrated that combining 4D-ELF- and LEBS-derived spectral markers enabled accurate identification of colonic neoplasia risk. Specifically, interrogation of the endoscopically normal rectum (either *ex vivo* or via fiberoptic probe) predicted the presence of advanced adenomas with $>$ 90% sensitivity, irrespective of location. Importantly, we have been able to achieve a negative predictive value of 100%, suggesting that we can confidently forego colonoscopy if rectal spectral markers are negative. Moreover, these spectral markers were accurate in identifying subjects with either a personal or family history of colonic neoplasia. Large-scale clinical trials are ongoing.

Our long-term goal is to allow the primary care physician, using a free-standing 4D-ELF/LEBS rectal probe, to accurately determine the need and intensity of colonoscopic screening.

In vivo peptide targeted confocal imaging of colonic dyslasia

Thomas Wang
Stanford University School of Medicine, Department of Medicine, Division of Gastroenterology, Stanford, CA 94305, USA

Patient morbidity and mortality associated with cancer originating from epithelia of hollow organs may be reduced by early detection of pre-malignant (dysplastic) mucosa. Phage display provides a high-complexity library of peptides which can be screened to identify high-affinity ligands that preferentially bind to molecular markers of disease. These peptides can be conjugated to fluorescence molecules, targeting dysplasia *in vivo*.

Selective peptides were identified using an M13 phage library by first clearing non-specific phage by biopanning against non-malignant human intestinal cells and fresh normal human colon biopsies. Unbound phage were subsequently panned using excised colonic adenomas. Promising peptides were sequenced, synthesized, tagged with fluorescein isothiocyanate (FITC), and administered topically onto a colonic adenoma and surrounding normal mucosa. After incubation for $\sim$1 minute, a prototype clinical confocal fluorescence microendoscope (CellVizio-GI, Mauna Kea Technologies, Paris, France) was used to image peptide binding at tissue depths of 0 to 50 :m. Imaging was performed at 12 frames per second with a resolution of 2.5–5 :m (transverse) and 15–20 :m (axial). Subjects were monitored for peptide toxicity by serum assays, liver function tests, and chemistries.

In vivo confocal fluorescence images were collected in nine patients undergoing routine colonoscopy. Imaging of fluorescence from FITC-conjugated peptide suggested binding to abnormal colonocytes. In contrast, reduced fluorescence was observed from surrounding normal mucosa from the same patients. Target-to-background ratios between adenomatous and normal colonocytes was $\sim$7 with an average signal-to-noise ratio of $\sim$5. No fluorescence was observed for non-peptide-treated mucosa. Imaged regions were removed by pinch biopsy and *in vivo* observations were confirmed by histopathology. No toxicity associated with peptide administration was found.

Dysplasia-binding peptides can be identified using phage display and selection using fresh human tissues, demonstrating the clinical potential of confocal microendoscopy to perform *in vivo* optical imaging of molecular targets.