Light-Scattering Technologies for Field Carcinogenesis Detection: A Modality for Endoscopic Prescreening

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Colonoscopy has revolutionized colorectal cancer (CRC) screening resulting in a decrease in both CRC mortality and incidence. Despite this, CRC still ranks as the second leading cause of cancer deaths among Americans, underscoring the need to both increase availability and accuracy of colonoscopy. The latter considerations provide the impetus for much of the current research into adjunctive imaging technologies. Recent advances in improving detection of dysplasia that have translated into clinical practice include high-definition scopes, narrow-band imaging, and chromoendoscopy. Another major direction of research into improving endoscopy is determining histology of lesions in situ ("optical biopsy") with confocal endomicroscopy, fluorescence, and elastic scattering spectroscopy. All these techniques are of great promise in improving delivery of endoscopy but, to date, have not addressed the potentially more important hurdle associated with logistic challenges of providing accurate CRC screening for the entire at-risk population.

The Need for Risk Stratification

The critical but heretofore relatively unexplored issue in endoscopic screening is the need for risk stratification. According to existing guidelines, every patient over the age of 50 is a candidate for colonoscopy.[1] However, the majority of the eligible population does not undergo recommended screening colonoscopies. Providing colonoscopy for the entire average-risk population (>100 million Americans over age 50) is impractical because of financial concerns (estimates of cost plus the economic impact range from $22 to $50 billion annually), resource constraints (insufficient number of endoscopists), complication rate (small but significant when applied to large populations), and patient noncompliance (owing to fear of complications, discomfort of the colonic purge, etc). This is juxtaposed with the fact that, in the average-risk population, the yield of screening-relevant neoplasia (advanced adenomas or early stage carcinomas) is remarkably low (~5%–6%). Even in patients with a personal history of adenomas (indication for ~20% of colonoscopies), the yields are low (~10%). Thus, >90% of the procedures do not engender cancer preventive ramifications (removal of significant lesions).[2]

To maximize the benefit to the population from the ~18–20 million colonoscopies performed in the United States annually, it has been advocated to increase the intervals between colonoscopies because most of the benefit from colonoscopic surveillance is derived from the first procedure.[3,4] Although justified from the societal point of view, from an individual's perspective, this is balanced by concerns of missed lesions (~5% of patients diagnosed with CRCs have had "negative" colonoscopies within the previous 3–5 years).[5] Furthermore, emerging evidence suggests that colonoscopy may be less effective (and possibly ineffective) in preventing right-sided CRCs.[6] These may be the central reasons behind the general overuse of colonoscopy with the negative consequences for cost, complications, and endoscopic capacity.

Thus, instead of a triage based simply on age or endoscopic findings, more accurate assessment of risk is urgently needed. Instead of performing colonoscopy on the entire population >50 years old, preselecting patients harboring significant lesions would allow the focusing of the finite endoscopic resource on subjects who would have a cancer preventive benefit (ie, undergo concurrent polypectomy; Supplementary Figure 1).[2] For a risk-stratification test to be practical, it has to be cost effective, have good patient acceptability, be performed by primary care physicians, and have a high sensitivity for significant lesions.

Field Carcinogenesis

For CRC screening, the rectum represents a site that could be both minimally intrusively interrogated and serve as a surrogate for neoplastic transformation elsewhere in the colon through the concept of field carcinogenesis (also known as field cancerization, field ef-

Abbreviations used in this paper: CRC, colorectal cancer; EIBS, early increase in blood supply; LEBS, low-coherence enhanced backscattering; PWS, Partial wave spectroscopic. ;

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fect, and field defect). Field carcinogenesis is a common theme in a variety of malignancies (colon, lung, pancreas, esophagus, stomach, ovarian, cervical, head and neck, liver, breast, prostate, etc).[7] It is the notion that the genetic/environmental milieu that leads to a focal tumor exists not only at that particular location, but affects the organ diffusely.[8] For instance, if a patient develops a cecal CRC, this occurred through interplay of both genetic and exogenous factors (eg, fecal stream mutagens) leading to stochastic mutations. Thus, the diffuse field changes provide a fertile mutational environment and predispose to carcinogenesis, while focal neoplastic lesions are determined by stochastic mutations. It follows that the uninvolved mucosa throughout the colon may serve as a surrogate site for assessing the risk of developing neoplasia with the rectum being the most readily accessible site.

Field carcinogenesis is well established in clinical practice. For instance, the distal adenoma found on flexible sigmoidoscopy portends a greater risk for synchronous proximal lesions, and thus mandates colonoscopy.[9] An adenoma on colonoscopy represents a higher risk of future neoplasia (metachronous lesions), thus providing the biological underpinnings behind postpolypectomy surveillance colonoscopy.[10] A number of other biomarkers of field carcinogenesis in endoscopically normal mucosa have been reported, including rectal aberrant crypt foci,[11] proliferation,[12] decreased apoptosis,[13] nuclear karyometry,[14] and genomic (microarray),[15] proteomic,[16] methylation,[17] transforming growth factor-α,[18] and crypt-restricted cytochrome C oxidase subunit I[19] markers. However, the diagnostic performance of current biomarkers has been suboptimal. These molecular changes would be anticipated to give rise to morphologic and functional alterations in colonic mucosa. Therefore, field carcinogenesis detection through evaluation of the endoscopically/microscopically normal rectal mucosa is biologically plausible. The central hurdle has been finding an accurate and practical biomarker, which is an emerging frontier for biophotonics.

Biophotonics Detects Multiple Facets of Field Carcinogenesis

The fact that the rectal epithelium in field carcinogenesis seems to be normal under light microscopy is related to the diffraction limit of resolution: It is not sensitive to structures <200–500 nm. Therefore, the intracellular structures that are dysregulated in early carcinogenesis (eg, mitochondria, higher order chromatin structure, and cytoskeleton) are not detectable by conventional light microscopy. Thus, there can still be profound functional, micro- and nanoarchitectural alterations in histologically normal epithelial cells undergoing field carcinogenesis. To detect these changes, we developed a suite of light-scattering technologies. There are several salient features of this platform, including ability to probe for structures at submicron scale, depth selectivity given the heterogeneous nature of the epithelium (eg, the earliest changes are believed to occur in proliferative/stem cells at the bottom third of the crypt), and ability to provide quantitative information. As opposed to imaging modalities that are able to visualize tissue structure but are qualitative, these approaches provide quantification of tissue/cell structure at submicron scales.

We focused on 3 facets of field carcinogenesis alterations, each representing a different level of tissue organization and a specialized technological solution for detection: (1) Physiologic targets such as microvascular blood content (reflecting the hyperproliferative state of the premalignant epithelium)[20–22]; (2) ultrastructural changes at the tissue level[23]; and (3) intracellular nanoarchitectural alterations[24–26] (Figure 1). All of these approaches provide the ability to accurately sense field carcinogenesis with slightly varying performance characteristics. There are, however, important differences in their clinical applications (in vivo measurements using fiberoptic probes versus analysis of cytologic slides from rectal brushings).

Increased Microvascular Blood Supply as a Marker of Field Carcinogenesis

Cells in field carcinogenesis are hyperproliferative and thus would be expected to be hypermetabolic. Thus, the logical corollary is that there is a need for an increased blood supply to the colonic epithelium. Previously, this has been difficult to detect because the pericryptal capillary plexus supplying blood to the epithelium represents only a very small portion of the total colonic blood supply and even marked changes in this compartment can be obscured by the rest of the vasculature.

Measuring blood content is a very well-studied application of biophotonics owing to the pathognomonic light absorption spectrum of hemoglobin. Because the increased microvascular blood supply in early colon carcinogenesis is expected to reside in the pericryptal capillary plexus surrounding the bases of the crypts (a few hundred microns below tissue surface), it is critical to restrict the depth of blood supply detection. Among other approaches, this can be accomplished through polarization gating, in which a tissue is illuminated by linearly polarized light and the difference between co- and cross-polarized reflected signals is generated primarily by short-traveling photons.[27] The depth of interrogation can be chosen from ~50 to a few hundred microns below the colonocytes depending on the design of a fiberoptic probe that is used for both illumination and collection of light interacting with the tissue (Figure 2*A*). Blood content is determined through the spectral analysis of the recorded signal. The measurable parameters include hemoglobin concentration, oxygenation, and average blood vessel diameter.

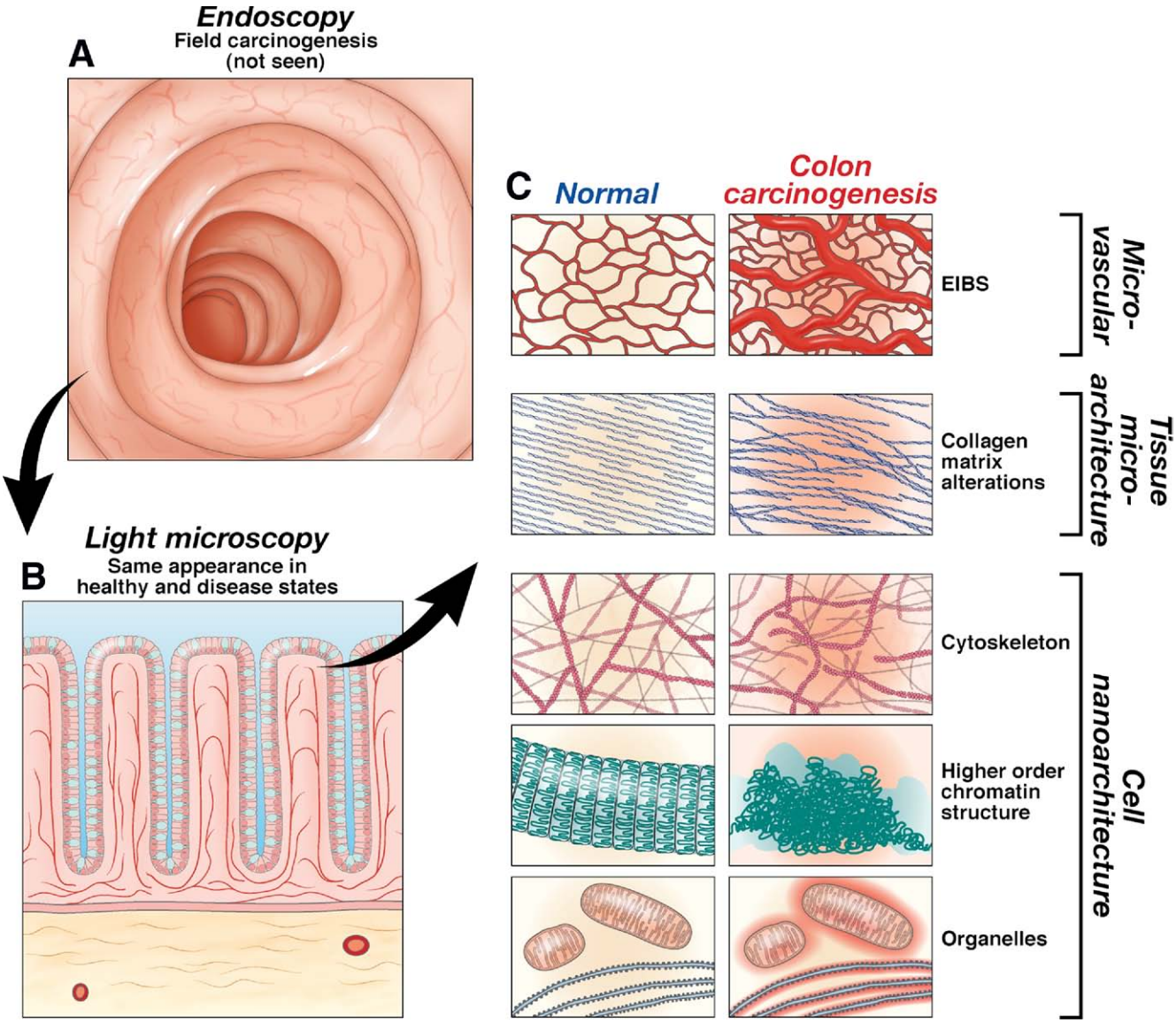
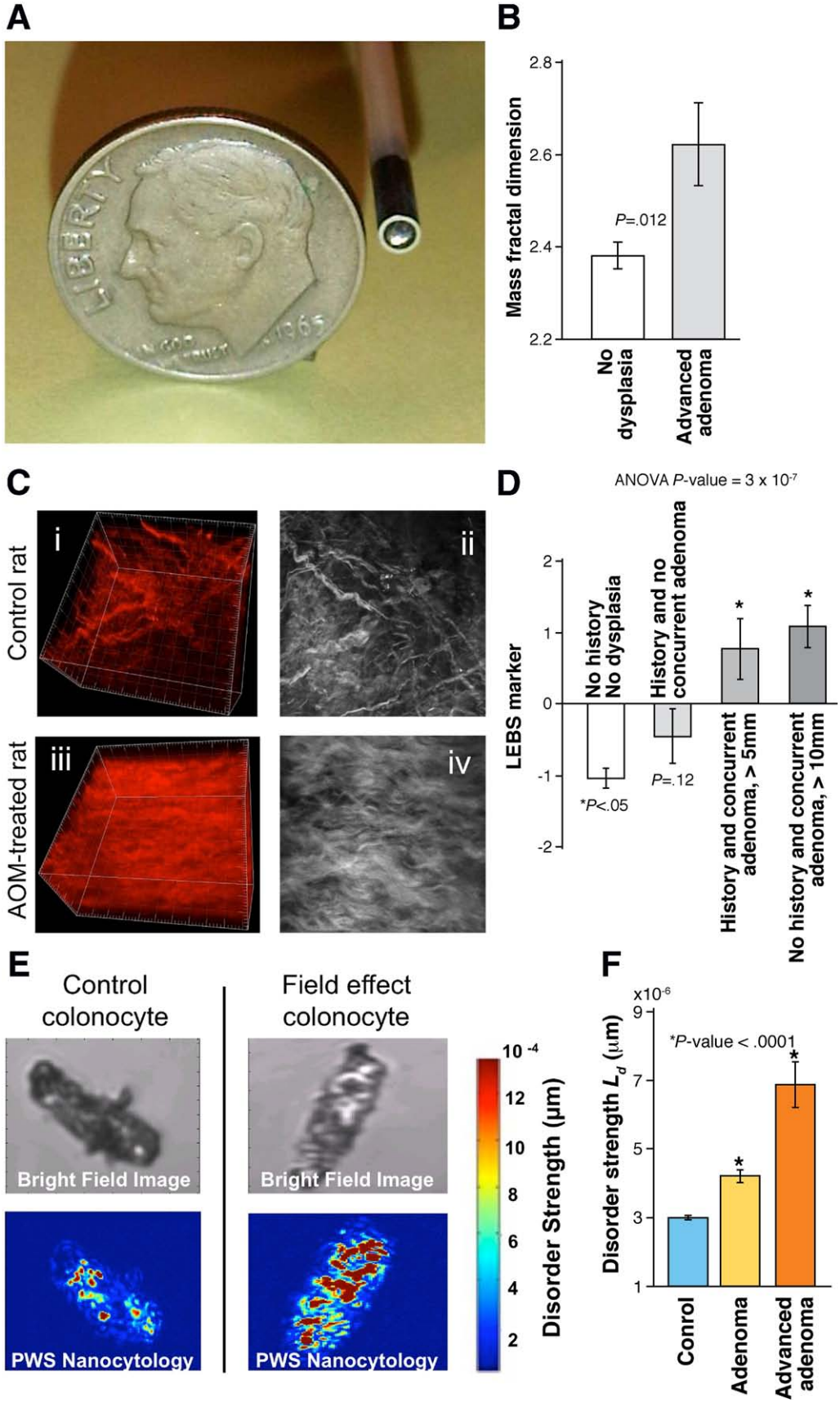


Figure 1. Field carcinogenesis has manifestations at a number of levels of tissue physiology and morphology that are not detectable by means of either endoscopy (*A*) or histopathology (*B*). These manifestations include alterations in mucosal microvasculature (detectable with a fiberoptic polarization-gated spectroscopy probe) and mucosal ultrastructure (detectable with LEBS and PWS microscopy) (*C*). The ultrastructural changes include alterations in the structure of extracellular matrix, cryptal architecture as well as the nanoscale architecture of colonocytes (eg, increase in the nuclear nanoscale disorder associated with alterations in the fractal organization of chromatin and chromatin compaction).

The phenomenon of increased microvascular blood content in early carcinogenesis [termed "early increase in blood supply" (EIBS)] was first observed in 2 animal models of CRC: The AOM-treated rat and the MIN-mouse with a germline APC mutation.[28] EIBS was detectable in the normal-appearing mucosa preceding formation of aberrant crypt foci and adenomas and progressed over time, paralleling the course of carcinogenesis. The phenomenon was largely confined to the mucosa.

EIBS is a robust marker of field carcinogenesis in humans.[20–22] This was confirmed in a study involving 222 unselected patients undergoing colonoscopy including 35 with nonadvanced and 12 with advanced adenomas. EIBS readings (each taking 50 milliseconds) were acquired in vivo by a fiberoptic probe from the endoscopically normal mucosa (cecum, mid transverse colon, and rectum). The data demonstrated that, in patients with neoplasia, EIBS was present diffusely throughout the colon. In addition, the magnitude of EIBS progressively increased when the measurements were taken closer to an adenoma. EIBS in the rectum was elevated irregardless of the location of the advanced adenoma.[22] The effect was the most pronounced close to the bases of the crypts (within ~100 μm below colonocytes). The area under the receiver–operator characteristic curve for advanced adenomas based on a single marker, mucosal oxygenated–


A
B
Mass fractal dimension
2.8
2.6
2.4
2.2
P=.012
No dysplasia
Advanced adenoma
C
Control rat
AOM-treated rat
i
ii
iii
iv
D
ANOVA P-value = 3 x 10-7
LEBS marker
2
1
0
-1
-2
No history No dysplasia
History and no concurrent adenoma
History and concurrent adenoma, > 5mm
No history and concurrent adenoma, > 10mm
*P<.05
P=.12
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E
Control colonocyte
Field effect colonocyte
Bright Field Image
Bright Field Image
PWS Nanocytology
PWS Nanocytology
10 -4
12
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8
6
4
2
Disorder Strength (µm)
F
x10-6
Disorder strength L_d (µm)
9
7
5
3
1
*P-value < .0001
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Conrol
Adenoma
Advanced adenoma

hemoglobin concentration, was 0.88 with 83% sensitivity and 82% specificity. EIBS was not confounded by demographic factors or benign colonic disease.

The biological basis of EIBS seems to involve an induction of neoangiogenesis that is most pronounced in the area adjacent to the bottom of the crypt (the proliferative compartment where teleologically one would expect greatest EIBS). Although there are numerous potential molecular drivers, inducible nitric oxide synthase is at least one important factor.[29]

Our data suggested a 2-component origin of EIBS: A diffuse component related to field carcinogenesis and a component related to factors elaborated by the tumor. This lends itself to a number of distinct applications: (1) risk stratification via detection of the field carcinogenesis component in the rectum and (2) guide to colonoscopy for adenoma detection via detection of the tumor-related gradient of EIBS. The latter has been facilitated by the development of real-time data analysis and a sensor that automatically triggers readings upon a probe's contact with the mucosa. The EIBS gradient in the proximity to adenomas could serve as a "red flag" technology identifying the 10–30 cm of the colon that is likely to harbor neoplasia and thus require increased scrutiny (eg, chromoendoscopy).

Ultrastructural Markers of Field Carcinogenesis

The genetic and epigenetic alterations of field carcinogenesis can lead to significant ultrastructural consequences. For instance, many early events in colon carcinogenesis (eg, APC, E-cadherin, Src)[30] interact with the cytoskeleton and would be expected to alter cellular ultrastructure. In turn, cellular ultrastructure is inherently linked to biochemical processes within the cell. Examples include the modulation of gene transcription by the higher-order chromatin structure, effects of macromolecular crowding on protein folding, and the regulation of gene expression by the extracellular matrix.

Because the optical refractive index is linearly proportional to the local density of macromolecules (eg, proteins, lipids, DNA), alterations in tissue/cell structure can be assessed by light scattering. Importantly, light-scattering approaches are sensitive to subdiffractional length scales: A scattering pattern becomes featureless only when the size of a structure falls below ~1/20th of the wavelength of light (~20 nm for visible light).[31] Although visualization of such small objects with microscopy is impossible, light scattering allows measuring of their statistical properties.

A comprehensive approach to describe tissue ultrastructure is via a mass-density correlation function, which quantifies how spatial correlation between structures depends on distance.[32] The function can be measured by a light-scattering technique, low-coherence enhanced backscattering (LEBS).[33,34] The depth of tissue interrogation can be controlled by varying the spatial coherence length of illumination from tens to hundreds of microns.[35] LEBS can determine the shape of the correlation function, the average amplitude, and the length scale of mass density variations. These parameters serve as the ultrastructural markers of field carcinogenesis.

The initial evidence of ultrastructural alterations in field carcinogenesis came from the LEBS analysis of colonic mucosa of the AOM-treated rat and the MIN-mouse models with ultrastructural changes developing diffusely throughout the colon before formation of aberrant crypt foci.[36]

The risk-stratification potential of rectal LEBS analysis has been tested in humans (n = 270)[23] (Figure 2*B*). The rectal ultrastructural alterations were sensitive to nondiminutive adenomas located elsewhere in the colon irrespective of adenoma location. The markers were progressively altered from patients with 5- to 9-mm adenomas to those with advanced adenomas, thus paralleling the risk of progression to CRC. LEBS performance for advanced adenomas showed 0.90 area under the receiver–operator characteristic curve with 100% specificity and 80% speci-

Figure 2. (*A*) A photograph of a fiberoptic probe for quantitative measurement of mucosal microvasculature (eg, EIBS). The probe is about 2 mm in diameter and can be used either as an endoscopically compatible device or a stand-alone device for detection of EIBS in rectal mucosa. (*B*) Fractal dimension of rectal mucosal microarchitecture is altered in patients harboring significant neoplasia (advanced adenomas) elsewhere in the colon. The measurements were obtained using LEBS from the rectal histologically and endoscopically normal-appearing mucosa. (*C*) Microscale organization of collagen matrix in the lamina propria and the upper submucosa is a marker of field carcinogenesis. The images were obtained by use of second harmonic generation microscopy performed on histologically normal-appearing colonic mucosa in the AOM-treated rat model of colon carcinogenesis. The top 2 panels (*i* and *ii*) show representative second harmonic generation images of collagen matrix structure from saline-treated control rats whereas the lower panels (*iii* and *iv*) show the matrix structure in the AOM-treated rats. The 2 left panels (*i* and *iii*) show 3-dimensional reconstructions and the panels on the right (*ii* and *iv*) show the images integrated over all depths. (*D*) Effect of past history of adenomas on rectal mucosal ultrastructural marker of field carcinogenesis. The ultrastructural marker was created as a linear combination of ultrastructural alterations measured with LEBS from histologically normal rectal mucosa. Patients with adenomas removed on a prior colonoscopy but with no concurrent adenomas (n = 14) had insignificantly elevated LEBS marker (P = .12) compared with patients with no prior history and no concurrent adenomas (n = 121), whereas patients with both prior history and concurrent adenomas (n = 14) had significantly elevated LEBS marker (P = .001). (*E*, *F*) Nanocytology enables detection of field carcinogenesis and colon cancer screening. Nanocytologic analysis of histologically normal colonocytes demonstrated that the disorder of their nanoscale architecture was a marker of field carcinogenesis. Nanocytology was performed by use of PWS microscopy (*E*). In the nucleus, the increased disorder is associated with altered higher-order chromatin structure. (*F*) Increased nanoscale disorder of rectal histologically normal colonocytes is a marker of adenomas located elsewhere in the colon.

ficity. There was no confounding by demographic, risk factors, or benign colon pathology. The insensitivity to diminutive adenomas is probably of minimal clinical implications. Although this study was performed on rectal biopsies, LEBS enables a fiberoptics implementation. An LEBS fiberoptic probe can be delivered in vivo without bowel purging. To date, we have performed it with a 3-mm probe through an anoscope in > 200 patients with diagnostic performance equivalent to that of the ex vivo analysis.

From a mechanistic perspective, there are 3 potential facets of the structural alterations: Ultrastructure of colonocytes and extracellular matrix and crypt reorganization. First, LEBS revealed that colonocytes' structure resembles a mass fractal.[32] This is consistent with other recent reports suggesting that key cellular components are fractal, including the chromatin.[37,38] A myriad of molecular processes can be dramatically affected by a shift in a fractal dimension including gene co-localization, co-expression, and diffusion of transcription factors.[39] LEBS showed that the fractal dimension is decreased in field carcinogenesis colonocytes—indicative of a more "disordered" cell organization.

Second, a profound reorganization of the collagen matrix occurs with an increase in the fractal dimension of the matrix. There have been numerous studies showing altered gene expression and methylation in the matrix. Optically, these changes have been demonstrated using second harmonic generation imaging in ovarian cancer.[40] We have replicated these findings in the AOM-treated rat model (Figure 2*C*). It is, however, not yet clear whether the matrix restructuring is initiated by preneoplastic colonocytes or whether it is a microenvironment phenomenon. Superimposed upon this are other facets including potential cryptal reorganization.[41] Taken together, these effects lead to an increase in the mass fractal dimension of the mucosa (Figure 2*B*).

Other complimentary light-scattering technologies have also been used to detect microarchitectural alterations in the colon, including Fourier-domain low-coherence interferometry (Wax et al[42]) and elastic scattering spectroscopy (Bigio et al;[43] Supplementary Material).

Nanocytology for Field Carcinogenesis Detection

Instead of using a fiberoptic probe (as in LEBS or EIBS), an alternate approach to the detection of ultrastructural, histologically unapparent cellular alterations is by means of the analysis of cell nanoarchitecture in cytologic samples from rectal brushings (hence the term nanocytology). Nanocytology parallels the conventional cytology, except that it analyzes the nanoscale as opposed to the micron-scale morphology and is quantitative.

Recently, partial wave spectroscopic (PWS) microscopy was developed to enable nanocytology.[24] By focusing on photons interacting with a cell substantially in 1 dimension, PWS is sensitive to essentially any length scale of density fluctuations (limited by the technical aspects of instrumentation). PWS measures a statistic of spatial mass density variations termed the disorder strength, which is related to the amplitude and the correlation length scale of the density variations.

The first demonstration of nanocytology was in CRC cell lines and animal models (the AOM-treated rat and the MIN-mouse): The disorder strength paralleled cell tumorigenicity in otherwise histologically indistinguishable cells.[24] In human studies (n = 35), PWS-enabled nanocytology performed on rectal brushings from the endoscopically normal mucosa before colonoscopy enabled accurate detection of colon field carcinogenesis (Figure 2*E*, *F*).[26] Rectal colonocytes' disorder strength was progressively increased with the magnitude of neoplasia that the patients harbored, from nonadvanced to advanced adenomas.

Multiple functional and genomic consequences would be expected to stem from this alteration. The disorder strength is increased in the entire cell, although the main effect seems to be in the nucleus. Experiments with pharmacologic cytoskeleton disruption showed that the cytoplasmic disorder increase is related to cytoskeletal changes.[44] The nuclear disorder increase seems to reflect chromatin compaction and decrease in its fractal dimension partially mediated by histone deacetylase activity. Chromatin compaction, in turn, is expected to affect locally multiple facets of genome regulation including the work for separation of a DNA double helix during pre-initiation of transcription, accessibility of DNA to transcription factors, DNA–histone interactions, and the diffusion of transcription factors and mRNA within the nucleus. The potential consequences of decreased chromatin fractality include gene de-localization and altered co-expression.

Just as field carcinogenesis is a ubiquitous cancer phenomenon, PWS nanocytology-detectable increased nanoscale disorder is not restricted to colon carcinogenesis and has been demonstrated in a number of other malignancies (pancreatic, esophageal, lung, and ovarian; Supplementary Material).[26]

Optical Detection of Field Carcinogenesis for Surveillance of Postpolypectomy, Family History, and Chemoprevention

Applications of biophotonics detection of field carcinogenesis may go beyond risk stratification (Supplementary Material for details). Approximately 20% of colonoscopies are performed after polypectomy surveil-

lance.[45] Colonoscopic surveillance is recommended because patients with an adenoma detected on an initial colonoscopy are at a greater risk of recurrent neoplasia (metachronous lesions). This is highly inefficient with ~90% of postpolypectomy colonoscopies being negative and, still, 0.3%–0.9% of patients undergoing polypectomy developing cancer within 3 years.[46] Our retrospective analysis showed that the rectal ultrastructural markers are indicative of the risk of adenoma recurrence[47] (Figure 2*D*). The ultrastructural and microvascular markers may help endoscopists to determine patient-specific screening intervals, particularly in patients with negative colonoscopies: A positive ultrastructural/microvascular marker assessed via an endoscopically compatible LEBS/EIBS probe during a negative colonoscopy may trigger a shorter interval.

Family history of CRC is another common indication for colonoscopy. Frequently, the genes involved and their penetrance are unknown; it is impossible, therefore, to ascertain whether a family member has acquired the predisposition and rationally tailor a screening strategy. Our data showed the ability of the microvascular/ultrastructural markers to detect future neoplastic risk in the MIN-mouse (murine model of FAP) at a pre-adenoma time point (EIBS/LEBS)[48] and in neoplasia-free Lynch syndrome patients with germline mutations in hMLH1 or hMSH2 (~60%–80% lifetime CRC risk; PWS nanocytology) suggesting that the nanoscale disorder increase was proportional to the long term risk of CRC.

Finding a reliable intermediate biomarker of the efficacy of chemoprevention is critical for more rapid clinical trials and to personalize therapy. Currently, it is difficult to gauge effectiveness without many years of therapy.[49] Because field carcinogenesis is an early event and exquisitely reflects risk, this has potential for rapid assessment of chemopreventive response. Our data in the AOM-treated rat model showed that short-term sulindac administration resulted in a dose-dependent normalization of the ultrastructural markers (eg, mass fractal dimension) providing the impetus for an ongoing Phase II trial.

Conclusion

Optically detectable biomarkers of field carcinogenesis represent a number of levels of tissue organization and function, including microvascular, mucosal ultrastructural, and intracellular nanoscale alterations. Optical detection of field carcinogenesis has the potential to allow individualization of screening regimens. We envision the use of the no bowel preparation–required rectal fiberoptic test (eg, LEBS) or nanocytology of cells brushed from the rectal mucosa in the primary care setting to determine the need for colonoscopy. The strength of these markers is their accuracy and ease of implementation. This would avoid unnecessary procedures and open up the limited endoscopy capacity. This pre-screen strategy is analogous to the Pap smear–colposcopy paradigm, which has relegated cervical cancer from the number 1 to the 14th cause of cancer deaths in women. Given that field carcinogenesis is a common theme in a variety of cancers, increased nanoscale disorder detectable by nanocytology may find broad applications as an initial screening tool for a wide range of malignancies (Supplementary Material).

Supplementary Material

Note: The first 5 references associated with this article are available below in print. The remaining references accompanying this article are available online only with the electronic version of the article. To access the remaining references, as well as additional online-only data, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:10.1053/j.gastro.2010.11.023.

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Reprint requests

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Conflicts of interest

The authors have made the following disclosures: Dr. Backman and Roy are co-founders and stock holders in American BioOptics LLC which has a license to the technology discussed.

Supplementary Materials

Optical Detection of Field Carcinogenesis for Postpolypectomy Surveillance

Our primary research focus has been on screening, but approximately 20% of colonoscopies are performed for postpolypectomy surveillance.[45] Colonoscopic surveillance is recommended because patients with an adenoma detected on an initial colonoscopy are at a significantly greater risk for recurrent neoplasia (metachronous lesions), although this is highly inefficient with ~90% of postpolypectomy colonoscopies being negative for screening relevant neoplasia. Existing guidelines stratify based on severity of lesions on the initial colonoscopy with advanced and multiple nonadvanced adenomas having colonoscopy repeated in 3 years, whereas a single, nonadvanced adenoma mandates a repeat procedure in 5–10 years.[10] However, recent studies have not replicated these findings, impugning the robustness of the guidelines. Finally, there is a concern that even in expert hands, 0.3%–0.9% of patients undergoing polypectomy will develop a cancer within 3 years.[46] This has profound clinical and medicolegal implications. These are some of the reasons that surveillance guidelines are frequently ignored by clinicians, which, some have argued, represents a major threat to resources available for health care maintenance.[50] We tested whether rectal mucosal ultrastructural markers are indicative of the risk of adenoma recurrence. Because of methodologic constraints, this study was performed retrospectively. Patients with a previous history but no concurrent adenomas ($n = 14$) had altered LEBS, although this did not reach significance ($P = .12$), whereas patients with both prior history and concurrent nonadvanced, nondiminutive adenomas ($n = 14$) had significantly elevated LEBS marker ($P = .001$)[47] (Figure 3*D*). This suggests that there might be a long-term effect of field carcinogenesis, but surveillance strategies still may allow identification of patients with metachronous neoplasia. The ultrastructural and microvascular markers may help endoscopists to determine patient-specific screening intervals, in particular in patients with negative index colonoscopies (eg, a positive ultrastructural/microvascular marker assessed during an otherwise negative colonoscopy via an endoscopically compatible LEBS/EIBS probe may trigger a shorter interval).

Family History of CRC and Field Carcinogenesis

Because microvascular and ultrastructural markers seem to be powerful in evaluating risk, they may be applicable to the clinically common scenario of management of neoplastic risk in patients with family history. Not only is this a common indication for colonoscopy, but, because the genes involved and their penetrance are commonly unknown, it is not possible to ascertain whether a particular family member has acquired the predisposition or the absolute increase in risk engendered. Thus, at present, it is difficult to rationally tailor a screening strategy. We therefore evaluated the ability of the microvascular/ultrastructural markers to detect future neoplastic risk in defined genetic conditions as a proof of concept. We noted that in the MIN mouse (murine model of familial adenomatous polyposis), at a pre-adenoma time point, there were profound differences in both EIBS and LEBS markers suggesting sensitivity of this approach to inherited risk.[48] Furthermore, pilot PWS nanocytology analysis from rectal brushings showed that the nanoscale disorder was significantly increased in patients who had germline mutations in hMLH1 or hMSH2 (Lynch syndrome genes engendering a ~60%–80% lifetime CRC risk) but have not yet developed adenomas/CRC. The magnitude of rectal disorder strength increase surpassed that seen in sporadic patients with advanced adenomas (3%–5% risk of malignant transformation per year),[51] suggesting that the nanoscale disorder increase was proportional to the long-term risk of CRC.

Optical Field Carcinogenesis Detection for Chemoprevention

Because EIBS, LEBS, and PWS markers are robust for neoplastic risk in the entire colon, we reasoned that it would be of interest to see if interventions that modify risk (ie, chemoprevention) also alter the microcirculatory and ultrastructural markers. Finding a reliable intermediate biomarker is critical not only for more rapid early phase clinical trials, but, more important, to personalize therapy. For instance, although aspirin is clearly effective at preventing neoplasia, it works in only ~30%–50% of patients and the optimal dose is unclear (potentially related to pharmacogenomic considerations). However, it is difficult to gauge effectiveness without many years of therapy, which exposes all patients to the risks of aspirin, whereas only a minority derives chemopreventive benefit. This is the major reason why the US Preventive Service Task Force did not recommend aspirin for chemoprevention, citing the harms outweighing the benefits.[49] Because field carcinogenesis is an early event and exquisitely reflects risk, this has potential for rapid assessment of chemopreventive response. In this regard, we evaluated premalignant AOM-treated animals. Data showed that short-term sulindac administration resulted in a dose-dependent normalization of the ultrastructural markers, particularly the mass fractal dimension. We have seen similar results with other chemopreventive agents providing the impetus for an ongoing Phase II trial and correlation with conventional and pharmacogenomic markers.

Complimentary Optical Technologies for Detection of Colon Field Carcinogenesis

Other light-scattering technologies can also be used to detect microarchitectural alterations. Wax et al[42] developed Fourier domain low-coherence interferometry for depth-resolved measurement of cell nuclear morphology in situ and showed in the AOM-treated rat model

that nuclear enlargement is a marker of colon field carcinogenesis.[42] Bigio et al[43] have developed elastic scattering spectroscopy fiberoptic probe as a point spectroscopic measurement technique, over a broad wavelength range (eg, 320–900 nm), which, depending on the fiberoptic geometry, relies on detection of diffusely or subdiffusely multiple scattered light in tissue. In a study involving 160 patients, the investigators demonstrated that a classification scheme based on a number of light-scattering properties measured by elastic scattering spectroscopy along with the principal component analysis of the scattering spectra were able to differentiate between measurements taken from colonoscopically normal tissue in patients with and without adenomas.[43]

Optical Techniques Detect Field Carcinogenesis in Other GI and non-GI Malignancies

Just as field carcinogenesis is a ubiquitous cancer phenomenon, optical changes in uninvolved histologically normal tissue have been observed in other malignancies. PWS nanocytology-detectable increase in the disorder of nanoscale cell structure is not restricted to colon carcinogenesis and has been shown to be a marker of local and extended field carcinogenesis in a wide range of malignancies.[26] In pancreatic cancer, the disorder strength increase in cells brushed from the histologically normal periampullary duodenal mucosa correlated with presence of pancreatic cancer, thus suggesting the feasibility of identifying patients with pancreatic cancer by means of duodenal brushing without the risks associated with interrogation of the pancreatic duct.[26]

Nanocytology may also find application as an adjunct to conventional cytology to improve its accuracy. In this vein, increased disorder was observed on pancreatic fine-needle aspiration cytology specimens from patients with pancreatic cancer.[25] Nanocytology had a 100% agreement with conventional (microscopic) cytology when the latter was true positive for pancreatic cancer. Importantly, however, nanocytology remained positive in patients with pancreatic cancer even when conventional cytology was negative or indeterminate with the sensitivity and specificity of nanocytology 83% and 100%, respectively. This finding is consistent with the notion that nanoscale alterations develop first in early carcinogenesis preceding cell-level microscale (nuclear atypia, etc) and then tissue-level macroscale (polyp/tumor formation, loss of normal tissue architecture) morphologic alterations.

Lung cancer provides another vivid example of an extended field carcinogenesis. Although curable at early stages, lung cancer is typically diagnosed based on symptoms that are a harbinger of advanced and hence incurable disease (5-year survival ~15%). This underscores need for effective screening of at-risk population (current/former smokers). Field carcinogenesis is well established in lung cancer with numerous techniques showing that the histologically normal bronchial mucosa distant to a neoplastic lesion is altered with genetic, epigenetic, and morphometric consequences. Furthermore, evidence suggests that the buccal mucosa is a "molecular mirror" for lung carcinogenesis.[52] Our data ($n = 135$)[53] demonstrated that PWS nanocytology performed on cells brushed from the histologically normal buccal mucosa could discriminate between cancer-free smokers versus lung cancer patients with an AUC of 0.85. This relationship was not confounded by age, smoking intensity, or other demographic or risk factors. Nanocytology was equally sensitive to different types (small versus non-small lung carcinomas) and subtypes (adenocarcinoma, squamous cell carcinomas) of lung cancer. Importantly, nanocytology was sensitive to early stage (stages I and II) lesions without the decrease in performance, consistent with the nature of the alteration as a predisposing event in lung carcinogenesis. A clinical implication of these results is the potential feasibility of PWS nanocytology performed on cells brushed from the buccal mucosa as a prescreening tool performed in the primary care setting to identify the subset of patients who may benefit from further screening such as low-dose CT or bronchoscopy.

Similar increases in the disorder strength was also observed in patients harboring esophageal adenocarcinoma (detected in proximal squamous epithelial cells) and ovarian cancer (cells brushed from the fallopian tubes, endometrium, and cervix). It is intriguing that the increase in the nanoscale disorder is a ubiquitous marker of the field carcinogenesis in different malignancies, thus indicating its biological significance in carcinogenesis.

Our team has also demonstrated LEBS-detectable microarchitectural alterations in the extended field carcinogenesis associated with pancreatic cancer.[54] The study involved 203 patients (84 healthy controls, 44 with pancreatic adenocarcinomas including 26 with resectable tumors, 26 with family history of pancreatic cancer, 29 with cysts, and 20 with pancreatitis, nonpancreatic malignancies or benign disease). Biopsies were obtained from endoscopically and histologically normal periampullary duodenal tissue. LEBS-derived ultrastructural markers were significantly altered in PC patients and some of them were partially altered in patients with mucinous cysts.[54] For an independent testing set, an AUC of 0.85 with 95% sensitivity and 71% specificity were obtained. The performance for discriminating healthy controls versus resectable adenocarcinomas and mucinous cysts were also good with AUCs of 0.88 and 0.79, respectively. Demographic and risk factors did not seem to confound the diagnostic performance. The markers were altered irrespective of the location of the tumor in the pancreas (head, body, tail). The markers' alteration dissipated at about 10 cm from the ampulla. This suggests that the field effect is strongest adjacent to the ampulla as would be predicted if pancreatic secretions or tumor-related factors were involved in the pathogenesis of duodenal alterations.

There have been a number of reports of optical field carcinogenesis detection in the esophagus. For squamous esophageal neoplasia, Wax et al.[42] developed angle-resolved low-coherence interferometry for the depth-resolved nuclear morphometry of epithelial cells. Angle-resolved low-coherence interferometry was used to measure the size of cell nuclei, their average density, and the fractal dimension of tissue. The latter was assessed for length scales greater than those probed by LEBS and PWS, that is, >1 micron. The technique was applied to an animal model of esophageal carcinogenesis. The investigators observed cell nuclear enlargement and an increased fractal dimension of tissue 50–100 microns beneath tissue surface in otherwise normal-appearing esophageal mucosa as a result of neoplastic transformation (induced by treatment with the carcinogen *N*-nitrosomethylbenzylamine). The alterations were mitigated by the action of 2 chemopreventive agents (difluoromethylornithine and perillyl alcohol).[55,56] The alterations were observed throughout the esophageal mucosa, thus indicative of field carcinogenesis. For esophageal adenocarcinoma, Periera et al applied elastic scattering spectroscopy in endoscopically normal Barrett's mucosa and were able to differentiate among patients with and without histopathologically confirmed high-grade dysplasia in Barrett's esophagus.[57]

In conclusion, given that field carcinogenesis is a common theme in a variety of cancers, increased nanoscale disorder detectable by nanocytology may find broad applications as an initial screening tool. Future studies will have to address the biological mechanisms of the ultrastructural and nanoscale alterations. Their relevance to a number of types of malignancies with very different genetic pathways suggests that these mucosal and cellular changes most likely play an important role in cancer progression. In this respect, certain parallels with the histological markers of neoplasia (ie, the most ubiquitous, albeit biologically still poorly understood) can be pointed out. Understanding the molecular mechanisms of these alterations and the interplay between structural, functional, and molecular events in field carcinogenesis may lead to new insights into early carcinogenesis.

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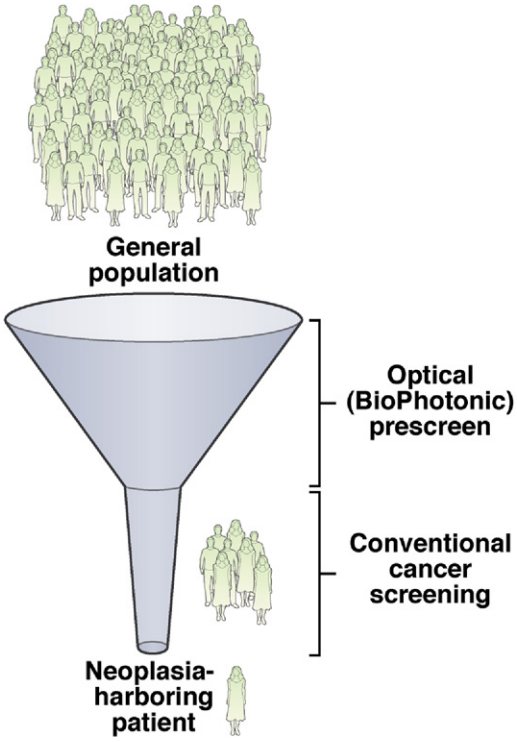

Supplementary Figure 1. Efficacious population screening for colon cancer would require risk stratification as a prescreen for colonoscopy that would identify a subset of the population at risk for harboring significant lesions who would benefit from colonoscopy.