Published in final edited form as:

Dis Colon Rectum. 2008 September ; 51(9): 1381–1386. doi:10.1007/s10350-008-9384-3.

Spectral Slope from the Endoscopically-Normal Mucosa Predicts Concurrent Colonic Neoplasia: A Pilot Ex-Vivo Clinical Study

Hemant K. Roy, M.D.¹, Vladimir Turzhitsky⁴, Young L. Kim, Ph.D.⁴, Michael J. Goldberg, M.D.¹, Joseph P. Muldoon, M.D.², Yang Liu, Ph.D.⁴, Randall E. Brand, M.D.¹, Curtis Hall, M.D.³, Nahla Hasabou¹, Mohammed Jameel¹, and Vadim Backman, Ph.D.⁴

¹ Department of Internal Medicine, Evanston-Northwestern Healthcare Evanston IL

² Department of Surgery, Evanston-Northwestern Healthcare Evanston IL

³ Department of Pathology, Evanston-Northwestern Healthcare Evanston IL

⁴ Biomedical Engineering Department, Northwestern University Evanston, IL

Abstract

Purpose—We previously reported that analysis of histologically normal intestinal epithelium for spectral slope, a marker for aberrations in nanoscale tissue architecture, had outstanding accuracy in identifying field carcinogenesis in preclinical colorectal cancer models. In this study, we assessed the translatability of spectral slope analysis to human colorectal cancer screening.

Methods—Subjects (n=127) undergoing colonoscopy had spectral slope determined from two endoscopically normal midtransverse colonic biopsies using four dimensional elastic light-scattering fingerprinting and correlated with clinical findings.

Results—Four dimensional elastic light-scattering fingerprinting analysis showed the submicron particles size progressively shifted towards larger sizes in subjects with harboring neoplasia. There was a corresponding decrease in spectral slope values from the endoscopically normal mucosa in subjects harboring adenomas (n=41) and advanced adenomas (n=10), compared to neoplasia-free subjects (p<0.00001). These factors did not appear to be confounded by either age or adenoma location. For detecting advanced adenomas, spectral slope had a negative and positive predictive value of 95 percent and 50 percent respectively.

Conclusions—We demonstrate, for the first time, that spectral slope in “normal” mucosa accurately risk-stratify patients for colonic neoplasia. This proof of concept study serves to underscore the promise of four-dimensional elastic light-scattering fingerprinting analysis for colorectal cancer screening.

Keywords

colon cancer; colon cancer screening; spectroscopy

Please address all correspondence and reprint requests to: Hemant K. Roy M.D., Associate Professor of Medicine, Feinberg School of Medicine at Northwestern University, Evanston Northwestern Healthcare Research Institute, Walgreen’s Building SB 521, 2650 Ridge Avenue, Evanston, IL 60201, h-roy@northwestern.edu, Phone: 847-570-3115, Fax: 847-733-5041.

Presented in part in abstract form at the 2006 Annual Scientific Meeting of the American Society of Colon & Rectal Surgeons in Seattle, WA, June 3 – 7, 2006.

Disclosures:

Drs. Roy, Goldberg and Backman are co-founders and stock holders in American BioOptics.

Dr. Roy had full access to the data and acts as the guarantor for the study.

Introduction

The lack of an inexpensive, minimally intrusive, and sensitive colorectal cancer (CRC) screening test has contributed to the continued high mortality rate from this malignancy in the United States.¹ Many CRC screening techniques are based on exploiting of the “field effect” concept of colon carcinogenesis.² This is the proposition that the genetic/environmental milieu that leads to neoplastic lesions in one area of the colon should be detectable, in some form, throughout the colon.³ Thus, assessing the easily accessible mucosa (*i.e.*, rectosigmoid) should potentially allow risk assessment of the entire colon. Indeed, several reports have demonstrated that there are profound genetic and cellular changes exist in the microscopically normal mucosa of patients who harbor CRC.⁴ However, the most-widely clinically utilized marker of the field effect, the distal adenoma detected by flexible sigmoidoscopy, lacks both adequate sensitivity and positive predictive value.^{5, 6} Therefore, finding accurate, practical means of identifying field changes would be of great clinical importance.⁷

Members of our group have pioneered the use of light-scattering technologies for detection of subtle cell structural changes during neoplastic progression.^{8, 9} Recently, we have developed four-dimensional elastic light scattering fingerprinting (4D-ELF) that allows heretofore impossible quantitative assessment of the nanoscale tissue architecture.^{10, 11} Information on particle sizes ranging from macromolecular complexes to small organelles has been harnessed through a spectroscopic parameter termed spectral slope. Our group has previously demonstrated that in the histologically normal colonic mucosa of the azoxymethane (AOM)-treated rat model, spectral slope was markedly altered prior to development of the earliest conventional markers of colon carcinogenesis such as aberrant crypt foci (ACF) or adenomas.¹⁰ Furthermore, we have reported that spectral slope from the “normal” intestinal mucosa of the MIN mouse (a genetic model that replicates familial adenomatous polyposis) was markedly suppressed prior to the occurrence of adenomas.¹² These findings with spectral slope clearly outperform any previous biomarkers for colon carcinogenesis.

In the present study, we explore the translatability of spectral markers to human colon carcinogenesis by assessing the spectral slope from the normal epithelium obtained during colonoscopy. We focus on spectral slope, our best validated markers in experimental models. We demonstrate that spectral slope was dramatically decreased in patients with colonic neoplasia (adenomas and advanced adenomas) when compared to subjects who were neoplasia-free providing a proof of principle that this biomarker may be able to risk stratify patients for colon neoplasia.

Materials and Methods

All studies were performed in accordance with the Institutional Review Board of Evanston-Northwestern Healthcare. Patients were eligible for the study if they were undergoing colonoscopy at Evanston Hospital for colon neoplasia detection (predominantly average risk screening with a smaller subset for evaluation of hematochezia of guaiac positive stools). The exclusion criteria included incomplete colonoscopy, poor preparation, coagulopathy, personal or family history of colon neoplasia or colitis. Two colonic biopsies were obtained from endoscopically normal midtransverse mucosa irrespective of findings on colonoscopy. However, we insured that all biopsies were taken at least 5 cm away from any neoplastic lesion. Biopsies were placed in phosphate buffered saline and subjected to 4D-ELF analysis. Approximately ten 4D-ELF measurements per subject were obtained. The histologic diagnosis was confirmed through independent review by a study pathologist (CH).

Advanced adenomas were defined as size ≥ 1 cm, >25 percent villous component or high-grade dysplasia).

Size Distribution Analysis and Spectral Slope Determination

4D-ELF analysis was performed by investigators blinded to clinical findings as previously described.^{10, 12} The spectra computationally simulated using Mie theory were fit to the spectra acquired by 4D-ELF after these were averaged over scattering angle from 0 to 5° and azimuth angle 0° using the conventional least-squares minimization algorithm. As previously reported, the log-normal size distributions provided fits superior to those obtained using other types of size distribution. The outcome of the fitting procedure was the determination of the mean size and the standard deviation of the tissue structures that gave rise to the scattering signal. Spectral slope was defined as the absolute value of the coefficient of the linear fit of spectral variations of spectral variation $\Delta I(\lambda)$ using linear regression analysis.^{10, 12}

Statistical Analysis

Values for spectral slope were analyzed using a two-sided Student's *t*-test. For the estimate of performance characteristics, patients with negative colonoscopies and non-advanced adenomas were divided equally into a training and a testing set. Given the small number of patients with advanced adenomas, we decided to train on nonadvanced adenomas and test on advanced adenomas. The training set had 59 patients whereas the testing set had 68 patients (58 controls/nonadvanced adenomas + 10 advanced adenomas). Thus, the threshold value of normal/abnormal was determined for negative colonoscopy vs. non-advanced adenoma and tested on the negative colonoscopy vs. advanced adenoma. The thresholds in the training set were designed to optimize sensitivity at the expense of specificity. There was no significant difference in age or gender distribution between the training and testing set.

Results

Patient Characteristics

We recruited 127 patients (66 males and 61 females), 86 patients were without neoplasia and 41 harbored adenomas. Ten of these patients were designated as having advanced adenomas (all qualifying based on size ≥ 1 cm). The mean $\pm$ standard deviation of age of patients who were neoplasia free, adenomas or advanced adenomas were 55.2 ± 9.0 , 58.3 ± 8.5 , 62.4 ± 12.1 respectively.

Intracellular Size Distribution

Representative size distributions of intracellular structures obtained from biopsies of the endoscopically-normal midtransverse colonic mucosa are demonstrated in Figure 1. When compared to those who were neoplasia free, patients who harbored adenomas had an increased propensity for larger sized particles (*e.g.* $>0.1 \mu\text{m}$) with a corresponding decrease in smaller particles (*e.g.* $<0.05 \mu\text{m}$). This trend was even more striking in patients harboring advanced adenomas.

Spectral Slope

Spectral slope was calculated as a marker for the heterogeneity of submicron particle size. As demonstrated in Figure 2, the spectral slope of subjects without neoplasia was 0.072 ± 0.004 . In patients with adenomas, this decreased to 0.039 ± 0.003 representing decreased relative proportion of small particles with a concomitant increase in the proportion of large particles ($p < 0.00001$ vs. control subjects). Moreover, in the subset of patients with advanced adenomas, this was further decreased to 0.023 ± 0.006 ($p < 0.00001$ vs. control subjects).

Effect of Adenoma Location and Patient Age on Spectral Slope

For spectral slope to be useful for CRC screening, it would be important for this marker from one segment of the colon to reflect neoplasia throughout the organ. We, therefore, evaluated the effect of location of adenoma on spectral slope. As can be seen in Figure 3, there was no evidence of diminution of the median spectral slope field based on distance from biopsy. Specifically, ANOVA analysis was not markedly different in patients with adenoma located in right colon, transverse colon or left colon (p value= 0.14). Moreover, there was a statistically significant difference in mean spectral slope from adenoma harboring patients irrespective of adenoma location when compared to adenoma-free patients (unadjusted paired t -test).

With regards to potential confounders, age was felt to be important given it is one of the most powerful CRC risk factors. As previously noted, neoplasia patients were slightly older than controls. When the subjects were stratified by colonoscopic findings (<60 vs. $\geq$ 60), there was no evidence of a relationship between spectral slope and increase age per se (p values of 0.372, 0.388 and 0.598 for patients who were neoplasia free, adenomas or advanced adenomas, respectively). Furthermore, ANOVA analysis of age compared to spectral slope was not statistically significant ($p=0.39$).

Performance Characteristics

We assessed performance ability of the spectral slope (assessed from the histologically-normal mucosa) to predict advanced adenomas. We chose advanced adenomas because these lesions are clearly clinically and biologically significant in contrast with most diminutive adenomas. Moreover, the colonoscopic miss rate is significant for small adenomas (~25 percent) but low for advanced adenomas (~2 percent).¹³ Using threshold positive/negative value derived from a training set on nonadvanced adenomas, we calculated that spectral slope had a sensitivity of 60 percent, specificity of 93 percent, positive predictive value of 50 percent and negative predictive value of 95 percent for advanced adenomas. Receiver operator characteristic curve is presented in Figure 4 comparing no dysplasia with advanced adenomas with the area under of the curve of 0.80. It needs to be emphasized that given the modest number of advanced adenomas ($n=10$) these performance characteristics should be simply a preliminary estimate.

Discussion

This is the first clinical demonstration of the ability of 4D-ELF analysis from one area of endoscopically normal mucosa to identify concurrent neoplasia elsewhere in the colon. This confirms the translatability of our previous work from experimental models to humans. The spectral slope was markedly suppressed in subjects who harbored colonic adenomas when compared to those who were neoplasia free. Importantly, the spectral slope suppression was unrelated to location of the lesion supporting the robustness of this marker for identifying the field effect. While the modest number of patient would lead us to emphasize the pilot nature of this data, it does serve as an important proof of principle confirmation of the approach.

The concept of field effect in colon carcinogenesis is well established. For instance, from a morphologic perspective, the number of rectal ACF correlated with adenomas and carcinoma occurrence in the proximal colon.¹⁴ There have been a number of reports demonstrating profound cellular alterations (*e.g.*, altered apoptosis and proliferation rates) in the histologically normal mucosa of patients harboring neoplasia.^{15, 16} From a genetic/epigenetic perspective, Chen and colleagues noted dramatic overexpression of proto-oncogenes such as cyclooxygenase 2 and osteopontin in both the tumors and the

histologically normal mucosa at some distance from tumors.⁴ Furthermore, other groups have noted that loss of Insulin Growth Factor-1 imprinting in the endoscopically normal mucosa accurately identified risk of colonic neoplasia.¹⁷ In addition, the proteomic profile from histologically normal mucosa from patients harboring colonic neoplasia has been recently shown to be quite distinct from those that were neoplasia free.¹⁸ These genetic/epigenetic alterations would be expected to have micro-architectural consequences; however, devising a reliable methodology has not, to date, been possible. 4D-ELF allows, for the first time, a practical means of probing cellular micro-architecture. We have reported in experimental models that the profound early changes in spectral markers in colon carcinogenesis can be reversed by targeting specific proto-oncogenes thereby supporting the genetic-microarchitectural relationship of the field effect.¹⁹

Our development of 4D-ELF has allowed us to heretofore unachievable quantification of submicron particle size through the use of spectral markers. As indicated from the size-distribution data (Fig. 1), there was an increase in larger particle sizes (*e.g.*, $> 0.05 \mu\text{m}$) with a concomitant decrease in smaller particles *e.g.*, $< 0.1 \mu\text{m}$) in patients who harbored adenomas and advanced adenomas when compared to those who were neoplasia free. Therefore, the corresponding spectral slope (which represents the heterogeneity in particle size) values were markedly reduced in these patients. This is consistent with our previous studies showing that altered size distribution preceded the conventional biomarkers of colon carcinogenesis in experimental models.^{10, 12} We need to reiterate that these measures were taken from the histologically normal mucosa and thus were not related to dysplasia.

Our data indicates that spectral slope was able to identify subjects at risk of concurrent colon neoplasia with reasonable accuracy. The performance characteristics of spectral slope for advanced adenomas were modest with a negative and positive predictive value of 95 percent and 50 percent respectively and an area under the ROC curve was 0.80. This single marker compares favorably to currently utilized clinical tests such as fecal occult blood tests and fecal DNA analysis which has a negative and positive predictive value for advanced adenomas/carcinomas of < 80 percent, and ~ 50 percent respectively.²⁰ From a risk-stratification perspective, the strong negative predictive ability is of paramount importance, because the consequence of a false-positive result is minimal (undergoing a colonoscopy which current guidelines state is an indicated procedure for the entire average risk populations) whereas having a false-negative could be clinically disastrous (subject does not undergo further testing and develops colon cancer). This initial report on a single spectral marker (encompassing < 5 percent of the information from light scattering signatures) supports the promise in clinical practice. It is likely that use of multiple spectral markers would improve diagnostic accuracy. Indeed, in experimental models, we have demonstrated that utilization of a panel of markers increased performance characteristics to > 95 percent.^{10, 12}

Limitations of this study include the modest number of patients and the lack of CRCs. Additionally, in any colonoscopy-based study, the potential for missed adenomas must be considered (estimated to be 27 percent).¹⁵ To mitigate this issue, we focused on advanced adenomas (where the detection rate is 98 percent) for calculation of performance characteristics.¹³ Another issue is that we only assessed spectral slope from a single colon region so the reproducibility in other areas (especially the more readily accessible rectal mucosa) needs to be explored in future larger studies. In this regard, it was reassuring that the spectral slope was decreased irrespective of location of the adenomas suggesting that assessment of the rectal mucosa may be informative about lesions throughout the colon. The need for biopsies may be undesirable for population screening; therefore future studies will employ a recently developed fiber-optic probe that can measure spectral slope *in situ* (unpublished data). Confounding by CRC risk factors is potential concern. Age is the most

powerful risk factor for sporadic colonic neoplasias², but as previously discussed, there was no evidence that age was a confounding factor on spectral slope values. We did not assess other CRC risk factor including obesity, tobacco or alcohol use²⁰; however, it is unlikely that these factors are important enough to bias our results. Finally, it needs to be emphasized that spectral slope represents a minute fraction of the information encoded in light-backscattering. It is likely that a panel of 4D-ELF markers could outperform the assessment of spectral slope alone. However, our data indicates that spectral slope by itself is a useful marker for advanced adenomas, although the modest numbers in this study means this data should be construed as pilot in nature.

In conclusion, our report serves as proof-of-principle that spectral markers from the “normal” colonic mucosa can predict neoplasia risk in humans. Given the outstanding performance in experimental models, this approach has promise for human CRC screening. Future studies will be conducted to assess the performance of a panel of spectral markers in different regions of the colon. Our long-term goal would envision the use of spectral markers for long-term risk-stratification, thus enabling the individualization of recommendations regarding the modality and intensity of CRC screening.

Acknowledgments

Supported by research grants from the National Institutes of Health (U01CA111257, R01CA112315 and R01EB003682), National Science Foundation (BES-0238903), and Coulter Foundation

The authors Drs. Eric Elton, Jonathan Horwitz, Mick Meiselman, Yolandra Johnson, Sonali Master and Eric Cohen for their assistance in patient recruitment and Ms. Beth Parker for manuscript preparation.

References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43–66. [PubMed: 17237035]
2. Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology* 2003;124:544–60. [PubMed: 12557158]
3. Braakhuis BJ, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of Slaughter's concept of field cancerization: evidence and clinical implications. *Cancer Res* 2003;63:1727–30. [PubMed: 12702551]
4. Chen L, Hao C, Chiu Y, et al. Alteration of gene expression in normal-appearing colon mucosa of APCmin mice and human cancer patients. *Cancer Res* 2004;64:3694–3700. [PubMed: 15150130]
5. Imperiale TF, Wagner DR, Lin CY, Larkin GN, Rogge JD, Ransohoff DF. Risk of advanced proximal neoplasms in asymptomatic adults according to the distal colorectal findings. *N Engl J Med* 2000;343:169–74. [PubMed: 10900275]
6. Schoenfeld P, Shad J, Ormseth E, et al. Military Colorectal Cancer Screening Trials Group. Predictive value of diminutive colonic adenoma trial: the PREDICT trial. *Clin Gastroenterol Hepatol* 2003;1:195–201. [PubMed: 15017491]
7. Regueiro CR. AGA Future Trends Committee report: Colorectal cancer: a qualitative review of emerging screening and diagnostic technologies. *Gastroenterology* 2005;129:1083–103. [PubMed: 16143145]
8. Backman V, Wallace MB, Perelman LT, et al. Detection of preinvasive cancer cells. *Nature* 2000;406:35–6. [PubMed: 10894529]
9. Backman V, Gopal V, Kalashnikov M, et al. Measuring cellular structure at submicrometer scale with light scattering spectroscopy. *IEEE J Sel Top Quantum Electron* 2001;7:887–93.
10. Roy HK, Liu Y, Wali RK, et al. Four-dimensional elastic light-scattering fingerprints as preneoplastic markers in the rat model of colon carcinogenesis. *Gastroenterology* 2004;126:1071–81. discussion 948. [PubMed: 15057746]

11. Roy HK, Iversen P, Hart J, et al. Down-regulation of SNAIL suppresses MIN mouse tumorigenesis: modulation of apoptosis, proliferation, and fractal dimension. *Mol Cancer Ther* 2004;3:1159–65. [PubMed: 15367710]
12. Roy HK, Kim YL, Wali RK, et al. Spectral markers in preneoplastic intestinal mucosa: an accurate predictor of tumor risk in the MIN mouse. *Cancer Epidemiol Biomarkers Prev* 2005;14:1639–45. [PubMed: 16030095]
13. van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ, Dekker E. Polyp miss rate determined by tandem colonoscopy: a systematic review. *Am J Gastroenterol* 2006;101:343–50. [PubMed: 16454841]
14. Takayama T, Katsuki S, Takahashi Y, et al. Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 1998;339:1277–84. [PubMed: 9791143]
15. Bernstein C, Bernstein H, Garewal H, et al. A bile acid-induced apoptosis assay for colon cancer risk and associated quality control studies. *Cancer Res* 1999;59:2353–7. [PubMed: 10344743]
16. Anti M, Marra G, Armelao F, et al. Rectal epithelial cell proliferation patterns as predictors of adenomatous colorectal polyp recurrence. *Gut* 1993;34:525–30. [PubMed: 8491402]
17. Cui H, Cruz-Correa M, Giardiello FM, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science* 2003;299:1753–5. [PubMed: 12637750]
18. Zisman A, Nickolov A, Brand R, Gorchow A, Roy H. Associations between the age at diagnosis and location of colorectal cancer and the use of alcohol and tobacco: implications for screening. *Arch Intern Med* 2006;166:629–34. [PubMed: 16567601]
19. Polley A, Mulholland F, Pin C, et al. Proteomic analysis reveals field-wide changes in protein expression in the morphologically normal mucosa of patients with colorectal neoplasia. *Cancer Res* 2006;66:6553–62. [PubMed: 16818627]
20. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA vs. fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004;351:2704–14. [PubMed: 15616205]

Figure 1.

Representative size distributions of subcellular submicron structures measured by four dimensional elastic light-scattering fingerprinting (4D-ELF) from the histologically normal mucosa in patients with and without concurrent neoplasia. In patients who harbored colonic adenomas, there was a change towards the presence of larger particle sizes and the magnitude progressively increased in patients with advanced adenomas

Figure 2.

Four dimensional elastic light-scattering fingerprinting (4D-ELF) spectral slope from the endoscopically normal mucosa is altered in patients with neoplasia. This histogram demonstrates progressive decrease in spectral slope in patients who harbor adenomas and advanced adenomas located throughout the colon when compared to those who were neoplasia-free.

Figure 3. Correlation between location of neoplastic lesion and the spectral slope in the endoscopically normal midtransverse colon. In adenoma, harboring patients, there was no significant difference in midtransverse colon spectral slope regardless of adenoma location.

Figure 4. Preliminary Receiver operator characteristics for advanced adenomas vs. no dysplasia using individual datapoints. The area under the curve (AUC) is 0.796.