

Molecular Pathogenesis of Dysplasia in Idiopathic Inflammatory Bowel Disease (IIBD)

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Bullet Points

- Current clinicopathologic risk factors of cancer risk in IIBD are insufficient.
- An optimal colonic IIBD risk factor should: 1) prevent cancer by distinguishing progressors from nonprogressors, and 2) uniformly involve rectal mucosa to obviate the major problem of sampling error in present-day surveillance.
- Molecular biomarkers, particularly preclonal genomic markers such as FISH, telomeres, and anaphase bridges, along with gene hypermethylation, in retrospective trials show promise towards these ideal biomarker goals.

Introduction:

IIBD confers an increased risk of intestinal cancer (1,2). Clinical management options are suboptimal and include lifelong intensive endoscopic biopsy surveillance versus prophylactic colectomy (3). These pose serious difficulties for patients and physicians alike, and they are inaccurate, and expensive (3). Considering that approximately 90% of IIBD patients will never develop neoplasia, the great majority of these efforts are wasted. Better understanding of the earliest steps of UC tumorigenesis may uncover improved diagnostic markers to target the subset of patients at highest risk for cancer. Of at least equal importance, such markers could also save the large majority with limited risk from unnecessary procedures. Several molecular biomarkers show promise toward this end and will be reviewed.

Existing Cancer Risk Markers in IIBD:

The existing risk factors for cancer in IIBD are clinicopathologic and include extensive disease of greater than eight years duration, coexistent primary sclerosing cholangitis, family history of colon cancer, and emerging data reimplicating the severity of inflammatory activity (1-7). Unfortunately,

these markers have poor sensitivities and specificities, and there remains a great need for improved detection of neoplastic risk in IIBD to prevent incurable cancer.

The current gold standard biomarker for cancer risk in IIBD, against which all new markers must be measured, is histologic dysplasia. Despite its reign as the gold standard, dysplasia also has serious flaws as a biomarker. The natural history of dysplasia in IIBD remains largely unknown, as none of the available longitudinal clinical series have obtained sufficient biopsies from the followed patients to achieve high degrees of confidence in the extent or grade of dysplasia (8). Observer variability by pathologists in the histologic diagnosis of dysplasia is an inescapable problem. This is due to the wide spectrum of morphologic changes in IIBD tumorigenesis, without precisely definable diagnostic cutoffs for the histologic categories. Similarly, distinction of neoplasia from inflammatory change adds considerably to the challenge.

Probably the most important problem with dysplasia as our gold standard biomarker of cancer risk is sampling error within the large surface area of the colonic mucosa. Using standard colonoscopy, an estimated minimum of 33 jumbo biopsies is required to detect dysplasia with even 90% confidence (9). Unfortunately, few clinicians take this many biopsies (10-11), rendering typical surveillance colonoscopy analogous to breast cancer screening via mammography of only a single breast. While recent studies show that most dysplastic lesions in IIBD are endoscopically visible on routine “white light” colonoscopy, a still highly significant percentage of dysplasias and even cancers in IIBD remain grossly undetectable by trained endoscopists, ranging from 12.1% to 38.6% in reported recent series (12-14). Chromoendoscopy, confocal endomicroscopy, trimodal imaging, and other new endoscopic methodologies show promise for improved targeting of

dysplasia with reduction of sampling error, but this remains an area of active investigation and is not yet standard practice (15-18).

A pancolonic mucosal biomarker would circumvent sampling error altogether. Further, a pancolonic abnormality would also necessarily involve the most distal rectal mucosa and would therefore be accessible to far less invasive means than full colonoscopy. Nothing approaching this goal is available clinically at present, but molecular biomarkers, along with improved endoscopic methods, are showing promise.

Molecular Markers of Cancer Risk in IIBD:

Most of what is known about genomic biomarkers in IIBD neoplasia derives from studies of UC. Little is known about Crohn's disease at present, so that the remainder of the discussion will focus on the available data in UC.

Genetic biomarkers have been clearly shown to predate morphologic dysplasia in UC tumorigenesis. Thus, genetic alterations are detectable in biopsies that remain negative for dysplasia and are at considerable distances from dysplasia. Thus, while UC cancers arise from pre-invasive dysplasias (9,19,20) these develop in still larger genetically abnormal fields that often exhibit aneuploidy (9,21), numerous different single gene alterations such as p53 alterations (22-25), microsatellite instability (MSI) (26-28), genomic and transcriptomic alterations detected high density array profiling (29-33), DNA fingerprinting alterations (34-36) and gene hypermethylation (37-40). Using these techniques, investigators have found that genomic, epigenetic and expression alterations are early steps in UC neoplasia that in some studies can even

be detected in non-dysplastic, diploid biopsies that are far distant from neoplasia. As such, these molecular changes predate morphologic neoplasia and show promise as biomarkers of neoplastic risk.

A limitation of the above assays is that their detection thresholds usually require clonal expansion of the targeted cell population being tested. This means that at least 20-50% or more of the tested cells must harbor the abnormality for detection. As such, clonal events are necessarily more advanced lesions than alterations that affect small or “preclonal” cell populations (<10% of cells) or even individual cells that such clonal assays could never detect. Further, few of the above markers have been found to involve sufficiently broad fields, particularly the rectum, to serve as ideal pancolonial screening assays.

An ideal mucosal screening assay for cancer risk in IIBD would not only predate incurable cancer and be objective, highly accurate, sensitive, specific and reproducible, but it would involve the entire colon. This latter feature would obviate sampling error as the single most important problem in present day UC colorectal cancer surveillance. ER gene hypermethylation, however, shows promise towards this goal, as it has been found to involve rectal mucosa in 42.2% of UC progressors versus 6.9% of UC non-progressors (38-39).

Theorizing that *non-clonal* genomic alterations might be even earlier and involve even broader colonic fields than the aforementioned genetic alterations, investigators have also sought to analyze non-clonal genomic alterations. Three additional non-clonal or pre-clonal alterations, namely fluorescence in-situ hybridization (FISH) to detect chromosomal gains and losses, telomere shortening assays, and anaphase bridge assays, have been investigated (41-45).

Of these preclonal genomic assays, fluorescence in-situ hybridization (FISH) shows the most promise to date, with near perfect sensitivity and specificity on ROC analysis with optimum threshold cutoff for distinguishing UC progressors (n=14) from UC non-progressors (n=15) (41-43). FISH was selected because of its ability to identify chromosomal alterations in single interphase cells or small subpopulations of non-clonally expanded cells typically representing much less than 10% of the total cell populations analyzed. FISH thus allows for the detection of infrequent and possibly random changes predating clonal expansion. Specific FISH probes were based on consistent chromosomal abnormalities from multiple UC progressors using the high density genomic scanning technique of comparative genomic hybridization (CGH) on non-dysplastic, diploid UC mucosa.

Similar to FISH, telomere shortening and anaphase bridge assays can also evaluate individual cells and are therefore able to detect pre-clonal and earlier alterations in UC neoplasia (42-45).

Telomeres shorten with oxidative stress and with cell proliferation, both of which are elevated in UC relative to normal colon (46-47). Telomere shortening promotes chromosomal end-to-end fusion that results in chromosomal breakage during cell division at anaphase with subsequent arm gains and losses in the daughter cells. Such chromosomal bridge-breakage-fusion cycles are documented by the formation of anaphase bridges, in which two anaphase daughter nuclei remain abnormally connected by a strand(s) of fused chromosome(s) during mitosis (47-51). Anaphase bridges can be enriched in cell preparations by flow cytometric sorting of dividing cells (G2+M phase of the cell cycle), with morphologic quantitation of bridged nuclei as the biomarker. ROC analyses of these two assays show only slightly lesser performance as biomarker to distinguish UC

progressors and non-progressors, again using largely single and rectal biopsies (42,43,45).

Thus, these three genomic pre-clonal assays, namely FISH, telomere shortening and anaphase bridge development, in UC neoplasia can separately identify progressors from normal control patients (41-43,45) and more importantly from non-progressor patients (42-43,45). More recently this biomarker panel measured singly or in combination from the same biopsy was also shown to differentiate well-characterized progressor and nonprogressor patients (43). Specifically, FISH over four combined chromosomes, or telomere length combined with anaphase bridges, yielded better discrimination of progressors and non-progressors than any assay separately. Additional statistical rigor of ROC analysis and FISH entropy calculations further documented that UC progressors can be distinguished from non-progressors. By ROC analysis, mean total entropy combining all four chromosomes yielded a highly promising 100% sensitivity and 92% specificity with optimum choice of threshold for distinguishing the tested cohort of UC progressors from long-term UC non-progressors (43).

The preclonal genomic alterations discussed above used 1-2 random forceps biopsy samples from UC progressors and non-progressors. These were obtained from the distal most (82.8% of tested samples were rectal) non-dysplastic mucosa available from colectomies with simultaneous diagnoses of dysplasia/cancer elsewhere (42,43). In fact, the tested non-dysplastic samples were located an average 28 cm from the patients' simultaneous cancers or dysplasias. Thus, the abnormal genomic field appears to involve a large area, if not the entire colon of the tested UC progressors. Such diffuse involvement is exciting, as it may fulfill, if true, an important requirement for the ideal biomarker on UC colonic mucosa. Diffuse distribution would eliminate

the sampling error that today is arguably the greatest problem facing the gold standard biopsy surveillance for histologic dysplasia. As such, these genomic biomarkers could have profound implications for future surveillance strategy in UC by preventing unnecessary colonoscopy in non-progressor while better identifying the highest risk potential progressor group who could benefit from intensive biopsy surveillance to detect dysplasia.

These retrospective studies demonstrate that the minority subset of UC patients (~10%) most likely to benefit from intensive cancer surveillance and prevention strategies can be separately identified from the much larger UC non-progressor majority (~90%). Conceivably, if the assays perform as well in an ongoing prospective validation NIH R01 trial, UC patients could be tested for these far less invasive genomic assays on minimal sampling limited to distal rectal mucosa. Full colonoscopic biopsy surveillance for dysplasia would be reserved for patients with diagnostic genomic alterations. Those without genomic alterations on periodic testing might be able to avoid colonoscopy altogether. Further prospective confirmation, reproducibility testing, and longitudinal analyses over time will be essential future investigations to assess the robustness of these assays, the optimal numbers and sites for biopsy sampling, and the timing of testing onset and testing intervals.

REFERENCES - MOLECULAR PATHOGENESIS OF IIBD NEOPLASIA

1. Ekblom A, Helmick C, Zack, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. *N Engl J Med* 1990;323:1228-1233.
2. Jess T, Loftus EV Jr, Velayos FS, et al. Risk factors for colorectal neoplasia in inflammatory bowel disease: a nested case-control study from Copenhagen county, Denmark and Olmsted county, Minnesota. *Am J Gastroenterol* 2007;102:829-836.
3. Itzkowitz SH, Present DH, Crohn's and Colitis Foundation of America Colon Cancer in IBD Study Group. Consensus conference: Colorectal cancer screening and surveillance in inflammatory bowel disease. *Inflamm Bowel Dis* 2005;11:314-21.

4. Brentnall TA, Haggitt RC, Rabinovitch PS, Kimmey MS, Bronner MP, Kowdley KV, Allyn Stevens, Crispin DA, Emond M, Rubin CE. Risk and natural history of colonic neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis. *Gastroenterology* 1996;110:331-338.
5. Shetty K, Rybicki L, Brzezinski A, et al. The risk of cancer or dysplasia in ulcerative colitis patients with primary sclerosing cholangitis. *Am J Gastroenterol* 1999;94:1643-1649.
6. Nuako KW, Ahlquist DA, Mahoney DW, et al. Familial predisposition for colorectal cancer in chronic ulcerative colitis: A case-control study. *Gastroenterology* 1998;115:1079-1083.
7. Gupta RB, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, Bodian C, Ullman T. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007;133:1099-1105.
8. Bronner MP, Goldblum JR. Progression of flat low-grade dysplasia to advanced neoplasia in patients with ulcerative colitis. *Adv Anat Pathol* 2004;11:225-226.
9. Rubin CE, Haggitt RC, Burmer GC, Brentnall TA, Stevens AL, Levine DS, Dean PJ, Kimmey M, Perera DK, Rabinovitch PS. DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. *Gastroenterology* 1992;103:1611-1620.
10. Bernstein CN, Weinstein WM, Levine DS, Shanahan F. Physicians' perceptions of dysplasia and approaches to surveillance colonoscopy in ulcerative colitis. *Am J Gastroenterol* 1995;90:2106-2114.
11. Ullman T, Croog V, Harpaz N, Sachar D, Itzkowitz S. Progression of flat low-grade dysplasia to advanced neoplasia in patients with ulcerative colitis. *Gastroenterology* 2003;125:1311-1319.
12. Blonski W, Kundu R, Lewis J, Aberra F, Osterman M, Lichtenstein GR. Is dysplasia visible during surveillance colonoscopy in patients with ulcerative colitis? *Scand J Gastroenterol* 2008;43:698-703.
13. Rutter MD, Saunders BP, Wilkinson KH, Kamm MA, Williams CB, Forbes A. Most dysplasia in ulcerative colitis is visible at colonoscopy. *Gastrointest Endosc* 2004;60:334-339.
14. Rubin DT, Rothe JA, Hetzel JT, Cohen RD, Hanauer SB. Are dysplasia and colorectal cancer endoscopically visible in patients with ulcerative colitis? *Gastrointest Endosc* 2007;65:998-1004.
15. Hurlstone DP, Sanders DS, Lobo AJ, McAlindon ME, Cross SS. Indigo carmine-assisted high-magnification chromoscopic colonoscopy for the detection and characterisation of intraepithelial neoplasia in ulcerative colitis: a prospective evaluation. *Endoscopy* 2005;37:1186-1192.
16. Kiesslich R, Goetz M, Lammersdorf K, Schneider C, Burg J, Stolte M, Vieth M, Nafe B, Galle PR, Neurath MF. Chromoscopy-guided endomicroscopy increases the diagnostic yield of intraepithelial neoplasia in ulcerative colitis. *Gastroenterology* 2007;132:874-882.
17. van den Broek FJ, Fockens P, van Eeden S, Reitsma JB, Hardwick JC, Stokkers PC, Dekker E. Endoscopic tri-modal imaging for surveillance in ulcerative colitis: randomized comparison of high-resolution endoscopy and autofluorescence imaging for neoplasia detection; and evaluation of narrow-band imaging for classification of lesions. *Gut* 2008;57:1083-1089.
18. Hurlstone DP, Kiesslich R, Thomson M, Atkinson R, Cross SS. Confocal chromoscopic endomicroscopy is superior to chromoscopy alone for the detection and characterization of intraepithelial neoplasia in chronic ulcerative colitis. *Gut* 2008;57:196-204.
19. Karlen P, Lofberg R, Brostrom O, et al. Increased risk of cancer in UC: A population-based cohort study. *Am J Gastroenterol* 1999;94:1047-1052.
20. Rutter MD, Saunders BP, Wilkinson KH, et al. Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. *Gastroenterology* 2006;130:1030-1038.
21. Löfberg R, Broström O, Karlén P, Ost A, Tribukait B. DNA aneuploidy in ulcerative colitis: reproducibility, topographic distribution, and relation to dysplasia. *Gastroenterology* 1992;102:1149-1154.
22. Leedham SJ, Graham TA, Oukrif D, McDonald SA, Rodriguez-Justo M, Harrison RF, Shepherd NA, Novelli MR, Jankowski JA, Wright NA. Clonality, founder mutations, and field cancerization in human ulcerative colitis associated neoplasia. *Gastroenterology* 2008 Nov7. [Epub ahead of print]
23. Nathanson JW, Yadron NE, Farnan J, Kinnear S, Hart J, Rubin DT. p53 mutations are associated with dysplasia and progression of dysplasia in patients with Crohn's disease. *Dig Dis Sci* 2008;53:474-480.
24. Noffsinger AE, Belli JM, Miller MA, Fenoglio-Preiser CM. A unique basal pattern of p53 expression in ulcerative colitis is associated with mutation in the p53 gene. *Histopathology* 2001;39:482-492.
25. Brentnall TA, Crispin DA, Rabinovitch PS, Haggitt RC, Rubin CE, Stevens AC, Burmer GC. Mutations in the p53 gene: an early marker of neoplastic progression in ulcerative colitis. *Gastroenterology* 1994;107:369-378.
26. Brentnall TA, Crispin DA, Bronner MP, Cherian SP, Hueffed M, Rabinovitch PS, Rubin CE, Haggitt RC, Boland

- CR: Microsatellite instability is present in non-neoplastic mucosa from patients with chronic ulcerative colitis. *Cancer Res* 1996;56:1237-1240.
27. Løvig T, Andersen SN, Clausen OP, Rognum TO. Microsatellite instability in long-standing ulcerative colitis. *Scand J Gastroenterol* 2007;42:586-591.
 28. Fujiwara I, Yashiro M, Kubo N, Maeda K, Hirakawa K. Ulcerative colitis-associated colorectal cancer is frequently associated with the microsatellite instability pathway. *Dis Colon Rectum* 2008;51:1387-1394.
 29. Watanabe T, Kobunai T, Toda E, Kanazawa T, Kazama Y, Tanaka J, Tanaka T, Yamamoto Y, Hata K, Kojima T, Yokoyama T, Konishi T, Okayama Y, Sugimoto Y, Oka T, Sasaki S, Ajioka Y, Muto T, Nagawa H. Gene expression signature and the prediction of ulcerative colitis associated colorectal cancer by DNA microarray. *Clin Cancer Res* 2007;13:415-420.
 30. Colliver DW, Crawford NP, Eichenberger MR, et al. Molecular profiling of ulcerative colitis-associated neoplastic progression. *Exp Mol Pathol* 2006;80:1-10.
 31. Willenbacher RF, Aust DE, Chang CG, Zelman SJ, Ferrell LD, Moore DH 2nd, Waldman FM. Genomic instability is an early event during the progression pathway of ulcerative-colitis-related neoplasia. *Am J Pathol* 1999;154:1825-1830.
 32. Kupka S, Schröder K, Porschen R, Borchard F, Gregor M, Blin N, Holzmann K. Comparative genomic hybridization analysis of chromosomal alterations in patients with long-standing ulcerative colitis. *Int J Oncol* 2001;19:489-494.
 33. Skacel M, Crispin DA, Rabinovitch PS, Casey G, Martin M, Brentnall T, Tubbs RR, Bronner MP: Array-based comparative genomic hybridization (array-CGH) detects frequent genomic alterations in non-dysplastic biopsies from ulcerative colitis (UC) patients with neoplasia. *Mod Pathol* 2004;17(S-1):130A.
 34. Chen R, Rabinovitch PS, Crispin DA, Emond MJ, Koprowicz KM, Bronner MP, Brentnall TA. DNA fingerprinting abnormalities can distinguish ulcerative colitis patients with dysplasia and cancer from those who are dysplasia/cancer free. *Am J Pathol* 2003;162:665-672.
 35. Chen R, Bronner MP, Crispin DA, Rabinovitch PS, Brentnall TA. Characterization of genomic instability in ulcerative colitis neoplasia leads to discovery of putative tumor suppressor regions. *Cancer Genet Cytogenet* 2005;162:99-106.
 36. Chen R, Rabinovitch PS, Crispin DA, Emond MJ, Bronner MP, Brentnall TA. The initiation of colon cancer in a chronic inflammatory setting. *Carcinogenesis* 2005;26:1513-1519.
 37. Wang FY, Arisawa T, Tahara T, Takahama K, Watanabe M, Hirata I, Nakano H. Aberrant DNA methylation in ulcerative colitis without neoplasia. *Hepatogastroenterology* 2008;55:62-65.
 38. Tominaga K, Fujii S, Mukawa K, Fujita M, Ichikawa K, Tomita S, Imai Y, Kanke K, Ono Y, Terano A, Hiraishi H, Fujimori T. Prediction of colorectal neoplasia by quantitative methylation analysis of estrogen receptor gene in nonneoplastic epithelium from patients with ulcerative colitis. *Clin Cancer Res* 2005;11:8880-8885.
 39. Fujii S, Tominaga K, Kitajima K, Takeda J, Kusaka T, Fujita M, Ichikawa K, Tomita S, Ohkura Y, Ono Y, Imura J, Chiba T, Fujimori T. Methylation of the oestrogen receptor gene in non-neoplastic epithelium as a marker of colorectal neoplasia risk in longstanding and extensive ulcerative colitis. *Gut* 2005;54:1287-1292.
 40. Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 2001;61:3573-3577.
 41. Rabinovitch PS, Dziadon S, Brentnall TA, Emond M, Crispin DA, Haggitt RC, Bronner MP. Pancolonic chromosomal instability precedes dysplasia and cancer in ulcerative colitis. *Cancer Res* 1999;59:5148-5153.
 42. O'Sullivan J, Bronner MP, Brentnall TA, Finley JC, Shen W-T, Emerson S, Emond MJ, Gollahon KA, Moskovitz AH, Crispin DA, Potter JD, Rabinovitch PS. Chromosomal instability is related to telomere shortening in a human preneoplastic disease (ulcerative colitis). *Nat Genet* 2002;32:280-284.
 43. Bronner MP, O'Sullivan JN, Rabinovitch PS, Crispin DA, Chen L, Emond MJ, Rubin CE, Brentnall TA. Genomic biomarkers to improve ulcerative colitis neoplasia surveillance. *Am J Pathol* 2008;173:1853-1860.
 44. Risques RA, Lai LA, Brentnall TA, Li L, Feng Z, Gallaher J, Mandelson MT, Potter JD, Bronner MP, Rabinovitch PS. Ulcerative colitis is a disease of accelerated colon aging: evidence from telomere attrition and DNA damage. *Gastroenterology* 2008;135:410-418.
 45. Kinouchi Y, Hiwatashi N, Chida M, Nagashima F, Takagi S, Maekawa H, Toyota T. Telomere shortening in the colonic mucosa of patients with ulcerative colitis. *J Gastroenterol* 1998;33:343-348.
 46. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. *Ann NY Acad Sci* 2000;908:99-110.
 47. Blackburn EH. Structure and function of telomeres. *Nature* 1991;350:569-573.
 48. Hande MP, Samper E, Lansdorp P, Blasco MA. Telomere length dynamics and chromosomal instability in cells

- derived from telomerase null mice. *J Cell Biol* 1999;144:589–601.
49. Artandi SE, et al. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* 2000;406:641–645.
 50. Bailey SM, Murnane JP. Telomeres, chromosome instability and cancer. *Nucleic Acids Res* 2006;34:2408-2417.
 51. Montgomery E, Wilentz RE, Argani P, Fisher C, Hruban RH, Kern SE, Lengauer C. Analysis of anaphase figures in routine histologic sections distinguishes chromosomally unstable from chromosomally stable malignancies. *Cancer Biol Ther* 2003;2:248-252.