

## NEOPLASTIC RISK ASSESSMENT IN BARRETT'S ESOPHAGUS

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Barrett's esophagus is an acquired metaplastic change that occurs in the distal esophagus secondary to chronic gastroesophageal reflux. The normal squamous epithelium is replaced by a columnar epithelium containing a mixture of cell types and architectural patterns resembling both gastric and intestinal mucosa. The three major types of epithelium include (a) a fundic type containing parietal cells and chief cells, (b) a junctional or cardiac type containing mucus-secreting columnar cells of the cardiac type, and (c) a distinctive specialized intestinal epithelium as indicated by the presence of goblet cells. It is this latter type of intestinalized epithelium that defines Barrett's esophagus.

Estimates of the prevalence of Barrett's esophagus suggest that this disorder may be seen in as many as 1 in 100 persons (1). It is rare in children (although it does occur), and increases in frequency among older individuals so that after the age of 60 it may be found in approximately 1% of people (2). Barrett's esophagus preferentially affects white males and it remains unclear as to why some patients with reflux do not develop Barrett's esophagus. For example, in Japan where reflux commonly occurs, Barrett's esophagus is relatively rare.

The significance of identifying Barrett's esophagus lies in the fact that this condition carries with it a risk for the development of esophageal adenocarcinoma. Carcinomas that arise in the setting of Barrett's esophagus are thought to develop as a part of a dysplasia-adenocarcinoma sequence similar to that seen in the patients with inflammatory bowel disease. Dysplasia is associated with, and often precedes, the development of invasive carcinomas in patients with Barrett's esophagus.

Many chronic inflammatory conditions are associated with an increased risk of neoplastic transformation. In the gastrointestinal tract, cancer risk is increased in patients with chronic inflammatory bowel disease. In the stomach, the inflammation associated with *Helicobacter pylori* infection predisposes to the development of carcinomas and lymphomas. When dysplasia develops in the setting of chronic inflammatory conditions, it is often widespread and multifocal, suggesting that neoplastic progression may result from abnormalities occurring over large regions of the affected mucosa. Recent studies suggest that these mechanisms may be at work in Barrett's esophagus as well, and that a field effect occurs over the entire Barrett's mucosa (3). These changes are likely attributable to an increased rate of genetic mutation facilitated by widespread chronic mucosal irritation, inflammation and regeneration. Concurrent effects of increased proliferation resulting from the repair of the damaged mucosa also contribute to genetic damage. Rapidly dividing cells are known to be at increased risk for undergoing mutation when compared to quiescent cells. Furthermore, cell proliferation is required to fix genetic damage within the cell population.

Cancer risk in patients with Barrett's esophagus progressively increases as the epithelium undergoes changes from Barrett's metaplasia to low grade dysplasia, high-grade dysplasia and ultimately invasive carcinoma. Therefore, patients diagnosed with

Barrett's esophagus undergo routine endoscopic surveillance so that precancerous dysplasia might be detected prior to the development of invasive cancer. There are several difficulties, however, in determining which patients will ultimately progress to cancer. First, diagnostic difficulties may exist in establishing the diagnosis of dysplasia in the first place. Problems a pathologist may face in establishing a diagnosis of dysplasia include difficulties relating to sampling error, the distinction of reactive changes from changes due to dysplasia, differences in observer interpretation of the diagnosis of dysplasia, and difficulties in differentiating high-grade dysplasia from invasive carcinoma. Requiring confirmation of a diagnosis of dysplasia by a second expert pathologist may help in eliminating some of these pitfalls. Second, even if one can confidently make a diagnosis of dysplasia, it is not certain that any given patient will continue to progress through the metaplasia-dysplasia-carcinoma sequence. Recent studies also suggest that computerized morphometric analyses may additionally aid in distinguishing differing grades of dysplasia (4).

Because of the difficulties in distinguishing between regenerative changes and dysplasia and because not all individuals with dysplasia develop carcinomas, numerous adjunctive tools have been applied to objectify these diagnoses or to identify those patients who will subsequently develop carcinomas. Although a huge number of studies have examined potential markers of neoplastic risk in Barrett's esophagus patients, only a few markers appear to have potential utility in this regard. These include DNA ploidy studies, expression of proliferation markers, and expression of the tumor suppressor proteins p53 and p16.

### **DNA Ploidy Studies**

Generally, the abnormalities in DNA ploidy correlate well with conventional histologic diagnoses of dysplasia and carcinoma, and might prove valuable as an adjunctive tool in the evaluation of patients with Barrett's esophagus (5-7). DNA aneuploidy increases as the histologic grade of the epithelium increases during the neoplastic progression (5). However, even specimens that are histologically negative or indefinite for dysplasia may contain aneuploid cells. Careful mapping studies demonstrate that early carcinomas arise within a single aneuploid population (5). In some patients, the same aneuploid abnormalities extend over large segments of the BE, suggesting that a single abnormal cellular clone may spread to involve large mucosal areas.

DNA analyses may be valuable in differentiating reactive atypia from dysplasia or cancer in a small biopsy because high levels of aneuploidy generally occur only in patients with high degrees of dysplasia or adenocarcinoma. A normal DNA pattern in a biopsy indefinite for dysplasia might provide reassurance that the lesion may not progress onward. In contrast, an abnormal ploidy pattern might prompt rebiopsy or more frequent endoscopic surveillance.

Such comparative histology and ploidy analyses, however, must generally be carried out using Feulgen staining and image analysis since flow cytometric DNA ploidy analysis and histologic analysis cannot be performed on the exact same tissue specimen. Flow cytometry requires sampling of mucosa adjacent to a histologically characterized focus of dysplasia. As a result, the tissue submitted for flow cytometry may not be representative of the tissue used for histologic examination. In addition,

most techniques employed in ploidy analysis are expensive and cumbersome, and are therefore difficult to implement as a part of routine daily practice.

### **Proliferation Markers**

Immunohistochemical staining for MIB-1, the Ki-67 proliferation antigen, shows gradually increasing expression in the Barrett's esophagus to esophageal adenocarcinoma sequence (8-14). MIB-1 expression has been reported in approximately 25% of cells in non-dysplastic Barrett's epithelium, and up to 87% of cells in adenocarcinoma. In addition, the pattern of MIB-1 expression is altered in dysplastic versus non-dysplastic mucosa. In Barrett's esophagus without dysplasia the cells expressing Ki-67 are limited to the bases of the crypts, whereas in dysplasia, the proliferating cells extend upward into the upper portion of the crypt and onto the mucosal surface. A recent study suggests that the combined use of MIB-1 and p53 staining may be of value in decreasing interobserver variation in the diagnosis of Barrett's associated dysplasia (15).

### **p53 Alterations**

The p53 gene is the most frequently mutated gene identified to date in human cancers, and is widely recognized as a tumor suppressor gene whose deletions and/or mutations are oncogenic. P53 is implicated in control of cell proliferation and differentiation, DNA repair and synthesis, and programmed cell death. P53 affects cell cycle arrest in the G1 phase in response to DNA damage, presumably allowing injured cells time to affect DNA repair before entering S phase. Loss of this checkpoint control could potentially result in replication of damaged DNA, and the generation of genomic instability in affected cells.

Most mutations in the p53 gene are point mutations that alter the half-life of the p53 protein within affected cells. As a result, this stabilized p53 protein is detectable by immunohistochemistry. p53 immunostaining does not identify all mutations, and sometimes p53 positivity can be seen with simple overexpression of the wild-type protein. However, p53 immunostaining is simple to perform, and is relatively fast and inexpensive in comparison with mutation analysis.

Alterations in p53 expression are common in esophageal adenocarcinomas and Barrett's associated high-grade dysplasia (reviewed in 16). p53 abnormalities may also be identified in low grade dysplasia and metaplastic Barrett's epithelium without dysplasia, albeit at a lower frequency (17-20). In addition, p53 alterations reportedly are seen with greater frequency among those patients who will ultimately progress to high-grade dysplasia or carcinoma (17). This finding has prompted some to suggest that p53 immunohistochemistry be used as a complementary test with histologic evaluation in the diagnosis of dysplasia in patients with Barrett's esophagus. However, it is important to note that some studies comparing p53 immunohistochemistry with p53 mutation analysis have demonstrated high rates of both false positive and negative results (21, 22). In addition, the staining characteristics of different p53 antibody clones vary. Finally, there remain patients without evidence of p53 abnormalities who progress to develop cancer, and there are some with p53 mutations who do not (23). Because of these difficulties, we very rarely use p53 immunohistochemistry in our evaluation of biopsies from patients with Barrett's esophagus.

## **p16 Inactivation**

Inactivation of the tumor suppressor *p16*, located on chromosome 9, is one of the most common abnormalities identified in human tumors. *P16* encodes a cell cycle regulatory protein that inhibits cyclin dependent kinases 4 and 6, preventing phosphorylation of the retinoblastoma gene product, Rb. This results in block of cell cycle progression in the G1-S phase. Inactivation of *p16* may occur as a result of mutation, homozygous deletion or methylation of the promoter of the gene. Allelic loss of *p16* is common in esophageal adenocarcinomas, and appears to be an early event in the Barrett's esophagus-dysplasia-adenocarcinoma sequence (24-26) that provides affected cells with a survival advantage (25, 26). In addition, *p16* LOH has been reported in 35% of biopsies from Barrett's patients without dysplasia (18). This finding suggests that *p16* loss may be a marker for patients at risk for later development of dysplasia or carcinoma. Loss of *p16* expression may be identified using immunohistochemistry, but the practical utility of this stain in evaluating Barrett's-associated dysplasia is unclear (27).

From all of these studies, it is clear that there is no single molecular marker that will suffice to allow us to predict who will or will not develop cancer in the setting of Barrett's esophagus. Carcinogenesis is a multi-step process that occurs as a result of alterations in many different genes. Therefore, it is likely that we will be required to develop panels of markers, which may, in differing combinations, allow us to predict neoplastic risk in individual patients. We have clearly not yet achieved this level of sophistication in our understanding of Barrett's-associated neoplasia. With additional large, long-term follow-up studies, however, we may someday reach this goal.

## References:

1. Cameron AJ. Epidemiology of Barrett's esophagus and adenocarcinoma. *Dis Esoph* 15:106-8, 2002.
2. Cameron AJ, Lomboy CT. Barrett's esophagus: age, prevalence and the extent of columnar epithelium. *Gastroenterology* 103:1454-8, 1992.
3. Sabo E, Beck AH, Montgomery EA, et al. Computerized morphometry as an aid in determining the grade of dysplasia and progression of adenocarcinoma in Barrett's esophagus. *Lab Invest* 86:1261-71, 2006.
4. Prevo LJ, Sanches CA, Galipeau C, Reid BJ. p53-mutant clones and field effects in Barrett's esophagus. *Cancer Res* 59:4784-7, 1999.
5. Reid BJ, Haggitt RC, Rubin CE, Rabinovitch PS. Barrett's esophagus: correlation between flow cytometry and histology in detection of patients at risk for adenocarcinoma. *Gastroenterology* 93:1-11, 1987.
6. Nakamura T, Nekarda H, Hoelscher AH, et al. Prognostic value of DNA ploidy and c-erbB-2 oncoprotein overexpression in adenocarcinoma of Barrett's esophagus. *Cancer* 73:1785-94, 1994.
7. Rabinovitch PS, Longton G, Blount PL, et al. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 96:3071-83, 2001.

8. Fujii T, Nakagawa S, Hanzawa M, et al. Immunohistological study of cell cycle-related factors, oncogene expression, and cell proliferation in adenocarcinoma developed in Barrett's esophagus. *Oncol Rep* 10:427-31, 2003.
9. Going JJ, Keith WN, Neilson L, et al. Aberrant expression of minichromosome maintenance proteins 2 and 5, and Ki-67 in dysplastic squamous epithelium and Barrett's mucosa. *Gut* 50:373-7, 2002.
10. Hong MK, Laskin WB, Herman BE, et al. Expansion of the Ki-67 proliferative compartment correlates with degree of dysplasia in Barrett's esophagus. *Cancer* 75:423-9, 1995.
11. Halm U, Tannapfel A, Breitung B, et al. Apoptosis and cell proliferation in the metaplasia-dysplasia-carcinoma sequence of Barrett's esophagus. *Hepato gastroenterology* 47:962-6, 2000.
12. Whittles CE, Biddlestone LR, Burton A, et al. Apoptotic and proliferative activity in the neoplastic progression of Barrett's oesophagus: a comparative study. *J Pathol* 187:535-40, 1999.
13. Rioux-Leclerc N, Turlin B, Sutherland F, et al. Analysis of Ki-67, p53 and Bcl-2 expression in the dysplasia-carcinoma sequence of Barrett's
14. Feith M, Stein HJ, Mueller J, Siewert JR. Malignant degeneration of Barrett's esophagus: the role of the Ki-67 proliferation fraction, expression of E-cadherin and p53. *Dis Esophagus* 17:322-7, 2004.
15. Lörinc E, Jakobsson B, Landberg G, Veress B. Ki67 and p53 immunohistochemistry reduces interobserver variation in assessment of Barrett's oesophagus. *Histopathology* 46:642-8, 2005.
16. Keswani RN, Noffsinger A, Waxman I, Bissonette M. Clinical use of p53 in Barrett's esophagus. *Cancer Epidemiol Biomarkers Prev* 15:1243-9, 2006.
17. Skacel M, Petras RE, Rybicki LA, et al. p53 expression in low grade dysplasia in Barrett's esophagus: correlation with interobserver agreement and disease progression. *Am J Gastroenterol* 97:2508-13, 2002.
18. Younes M, Ertan A, Lechago LV, et al. p53 protein accumulation is a specific marker of malignant potential in Barrett's metaplasia. *Dig Dis Sci* 42:697-701, 1997.
19. Novotna K, Trkova M, Pazdro A, et al. TP53 gene mutations are rare in nondysplastic Barrett's esophagus. *Dig Dis Sci* 51:110-3, 2006.
20. Suspiro A, Pereira AD, Afonso A, et al. Losses of heterozygosity on chromosomes 9p and 17p are frequent events in Barrett's metaplasia not associated with dysplasia or adenocarcinoma. *Am J Gastroenterol* 98:728-34, 2003.
21. Ireland AP, Clark GW, DeMeester TR. Barrett's esophagus: the significance of p53 in clinical practice. *Annals Surg* 225:17-30, 1997.
22. Greenblatt MS, Bennett WP, Hollstein MC, et al. Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res* 54:4855-78, 1994.
23. Terano A, Morita K, Nakamura T, et al. Barrett's esophagus. *J Gastroenterol* 37:685-90, 2002.

24. Schulmann K, Sterian A, Berki A, et al. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 24:4138-48, 2004.
25. Maley CC, Galipeau PC, Li X, et al. Selectively advantageous mutations and hitchhikers in neoplasms: p16 lesions are selected in Barrett's esophagus. *Cancer Res* 64:3414-27, 2004.
26. Wong DJ, Paulson TG, Prevo LJ, et al. p16<sup>INK4a</sup> lesions are common, early abnormalities that undergo clonal expansion in Barrett's metaplastic epithelium. *Cancer Res* 61:8284-9, 2001.
27. Merola E, Mattioli E, Minimo C, et al. Immunohistochemical evaluation of pRB2/p130, VEGF, EZH2, p53, p16, p21<sup>waf-1</sup>, p27, and pCNA in Barrett's esophagus. *J Cell Physiol* 207:512-9, 2006.