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The Molecular Basis of Carcinogenesis in Barrett's Esophagus

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Our current management of cancer risk in Barrett's esophagus is to perform endoscopic surveillance for the detection of dysplasia. However, dysplasia is an imperfect predictor of cancer risk for a variety of reasons including biopsy sampling error, poor intra- and inter-observer reproducibility of dysplasia interpretations and the poor predictive value for negative, indefinite, low-grade, and even high-grade dysplasia.^{1–3} Dysplasia is a conglomerate of histologic abnormalities that suggest that clones of cells have acquired neoplastic properties that predispose them to cancer formation. Therefore, dysplasia is a surrogate marker for cells that have accumulated enough genetic damage that they now possess some of the physiologic properties of cancer cells. Therefore, a better indicator of cancer risk would be detection of the genetic damage itself before the histologic manifestations of dysplasia are even apparent. In addition, the identification of molecular biomarkers may offer easy reproducibility and standardization in addition to the truly early detection of neoplastic progression.

In the traditional phenotypic model, carcinogenesis in Barrett's esophagus is viewed as occurring in discrete steps from metaplasia to dysplasia and finally to carcinoma. In the genetic model, neoplastic progression is envisioned as a continuum over which cells progressively accumulate genetic abnormalities until they acquire the six essential physiologic hallmarks of cancer.⁴ These cancer hallmarks include the ability of cells (1) to provide their own growth signals, (2) to avoid growth inhibitory signals, (3) to avoid apoptosis, (4) to replicate without limit, (5) to sustain angiogenesis (the formation of new blood vessels), and (6) to invade and metastasize. These hallmarks represent the physiologic traits that must be acquired by cells during the genesis of all human tumors and, therefore, are not specific for neoplastic progression of Barrett's esophagus. However, there are differences among human tumors regarding the specific genetic alterations acquired that endow the cell with each of these physiologic hallmarks and we will highlight some of the genetic alterations that occur in Barrett's esophagus which allow the cell to acquire each of the hallmarks.

Hallmark 1: The Ability to Provide Growth Signals

In general, this occurs by the activation of oncogenes. Genes that stimulate cell growth in normal cells are termed protooncogenes. When these same genes become overactive as a result of certain types of mutations, they are called oncogenes. Thus, oncogene activation leads to

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uncontrolled cell growth. Examples of oncogenes implicated in Barrett's carcinogenesis include cyclins D1 and E, transforming growth factor- α , epidermal growth factor, and the epidermal growth factor receptor.⁵

Hallmark 2: The Ability to Avoid Growth Inhibitory Signals

In general, growth inhibitory signals are transmitted by tumor suppressor genes. Tumor suppressor genes are normal genes that restrain cell proliferation. When tumor suppressor genes are inactivated, the cells are able to avoid growth inhibitory signals allowing for uncontrolled proliferation. Mutation, deletion of the chromosomal region containing the gene (called loss of heterozygosity (LOH)), and attachment of methyl groups to the promoter region of genes (called promoter hypermethylation) are ways in which tumor cells can inactivate tumor suppressor genes. Examples of tumor suppressor genes inactivated during neoplastic progression of Barrett's esophagus include p53, p16, and the adenomatous polyposis coli (APC) gene.⁵

Hallmark 3: The Ability to Avoid Apoptosis

Apoptosis is a pre-programmed mechanism for normal cells to self-destruct. This is beneficial to normal cells, in that it prevents cells with damaged, mutated DNA from undergoing replication. However, to cancer cells, apoptosis is detrimental, and cancer cells must find ways to avoid self-destruction. Barrett's cells have found a variety of ways to overcome triggering apoptosis. For example, as already discussed, inactivation of p53 is one way in which Barrett's cells avoid inducing apoptosis in response to DNA damage or mutation. Another way Barrett's cells avoid apoptosis is by the upregulation of cyclooxygenase-2, a gene whose protein product exerts antiapoptotic effects. Finally, Barrett's cancer cells have been found to express Fas-ligand, a death-promoting ligand that can activate the apoptotic cascade within the tumor killing immune cells resulting in their destruction.^{6,7}

Hallmark 4: The Ability to Replicate without Limit

Normally, as cells undergo successive cell divisions, they reach senescence. Senescence is an intrinsic mechanism of cells that limits their normal proliferative capacity. The triggering of senescence involves the loss of telomeres which are repetitive pieces of DNA located at the ends of chromosomes. When telomeres become too short, senescence ensues. Telomerase is the enzyme that allows for the synthesis and stabilization of telomeres.⁸ Stable telomeres confer immortality to the cell. In contrast to normal esophageal tissues, benign Barrett's esophagus expresses low levels of telomerase, which appears to increase as the metaplastic cells progress to high-grade dysplasia and cancer.⁹

Hallmark 5: The Ability to Sustain Angiogenesis

In order for a tumor to increase in size, it must maintain an adequate blood supply. The synthesis of new blood vessels is termed angiogenesis. One way in which tumor cells synthesize new blood vessels is by secreting vascular endothelial growth factors (VEGFs) which promote the proliferation and migration of endothelial cells upon binding to their receptors, the vascular endothelial growth factor receptors (VEGFRs). The expression of VEGFs and VEGFRs has been found in metaplastic Barrett's esophagus as well as in neoplastic Barrett's tissues.^{10,11}

Hallmark 6: The Ability to Invade and Metastasize

Although the mechanisms of cancer cell invasion and metastasis are poorly understood, abnormalities in cell-cell interaction mediated by cadherins and catenins are thought to play a role.¹² In the neoplastic progression of Barrett's esophagus, the normal membranous location of E-cadherin and β -catenin decreases, and the cytoplasmic and nuclear staining for these proteins increases as the degree of dysplasia increases.¹³ In addition, Barrett's cancers

have been found to express matrix metalloproteases which degrade the extracellular matrix and facilitate invasion.¹⁴

Status of Biomarkers to Predict Cancer Risk in Barrett's Esophagus

Some of the individual abnormalities described above have been proposed as biomarkers for cancer risk in Barrett's esophagus, and it is likely that a few of these will eventually become clinically useful. The National Cancer Institute's Early Detection Network has proposed five phases of study that biomarkers must undergo for validation.¹⁵ It is only in the latter three phases that clinical studies are carried out to (1) evaluate retrospectively the predictive ability of the biomarker and to define a "positive" test (phase 3), (2) prospectively determine the predictive ability of the biomarker (phase 4), and (3) estimate the reduction in mortality by action taken based on the biomarker assay (phase 5).¹⁶ The majority of the biomarkers proposed for Barrett's esophagus have been evaluated in phase 3 studies, and none of these potential biomarkers have been evaluated in phase 5 studies. The Barrett's biomarkers that have shown the most promise thus far include aneuploidy and increased tetraploidy, 17p LOH, and 9p LOH.

Aneuploidy and Increased Tetraploidy

Aneuploidy does not indicate a single genetic abnormality but rather refers to an alteration in the normal diploid (2n) or tetraploid (4n) (where n refers to the number of chromosomes) DNA content of a cell. Thus, aneuploidy reflects the accumulation of multiple genomic abnormalities like the ones discussed above. Aneuploidy can be detected by flow cytometry or by fluorescence in situ hybridization (FISH); however, FISH may be less sensitive than flow cytometry in the detection of chromosomal abnormalities.^{17,18} A number of large prospective studies have found that aneuploidy and/or increased tetraploidy (>6% of cells within a tissue with 4n) are significant predictors of cancer risk in Barrett's esophagus.^{3,19,20}

17p LOH

17p is the chromosomal locus for p53, and a number of studies have investigated the ability of 17p LOH to predict neoplastic progression of Barrett's esophagus.^{17,21–23} In two large prospective studies 17p LOH, as detected by flow cytometry, in baseline biopsy specimens of Barrett's esophagus was found to be a significant predictor of neoplastic progression regardless of the degree of dysplasia.^{21,22} More recently, a number of cross-sectional studies have reported promising results on the ability of 17p LOH, detected by FISH in biopsy tissues and in brush cytology specimens of Barrett's esophagus, to predict neoplastic progression.^{17,23}

Aneuploidy/Increased Tetraploidy, 17p LOH, and 9p LOH in Combination

In a large, prospective study, the ability of this combination of biomarkers to predict neoplastic progression in Barrett's esophagus was found to be better than any of the biomarkers used alone.²² The incidence of cancer was 80% at 6 years in those patients whose biopsies contained all three abnormalities, whereas the incidence of cancer was 12% at 10 years in those patients whose biopsies did not demonstrate any of these abnormalities.²² Thus, it is likely that a panel of biomarkers will be better predictors of neoplastic progression in Barrett's esophagus than a single individual genetic abnormality.

Overall, although the results of these studies are promising, the use of these biomarkers in routine clinical practice is not yet recommended. In light of the recent advances in biomarker discovery, it is likely that combinations of molecular biomarkers will eventually be better predictors of neoplastic progression than dysplasia. Large, prospective clinical trials of candidate biomarkers (phases 4 and 5 studies) for detecting cancer arising in Barrett's esophagus are eagerly awaited.

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References

1. Reid BJ, Haggitt RC, Rubin CE, Roth G, Surawicz CM, Van Belle G, Lewin K, Weinstein WM, Antonioli DA, Goldman H. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 1988;19:166–178. [PubMed: 3343032]
2. Montgomery E, Bronner MP, Goldblum JR, Greenson JK, Haber MM, Hart J, Lamps LW, Lauwers GY, Lazenby AJ, Lewin DN, Robert ME, Toledano AY, Shyr Y, Washington K. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol* 2001;32:368–378. [PubMed: 11331953]
3. Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 2000;95:1669–1676. [PubMed: 10925966]
4. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70. [PubMed: 10647931]
5. Souza RF, Morales CP, Spechler SJ. Review article: a conceptual approach to understanding the molecular mechanisms of cancer development in Barrett's oesophagus. *Aliment Pharmacol Ther* 2001;15:1087–1100. [PubMed: 11472311]
6. Suda T, Takahashi T, Golstein P, Nagata S. Molecular cloning and expression of the Fas ligand, a novel member of the tumor necrosis factor family. *Cell* 1993;75:1169–1178. [PubMed: 7505205]
7. Younes M, Schwartz MR, Ertan A, Finnie D, Younes A. Fas ligand expression in esophageal carcinomas and their lymph node metastases. *Cancer* 2000;88:524–528. [PubMed: 10649242]
8. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997;33:787–791. [PubMed: 9282118]
9. Morales CP, Lee EL, Shay JW. In situ hybridization for the detection of telomerase RNA in the progression from Barrett's esophagus to esophageal adenocarcinoma. *Cancer* 1998;83:652–659. [PubMed: 9708927]
10. Couvelard A, Paraf F, Gratio V, Scoazec JY, Henin D, Degott C, Flejou JF. Angiogenesis in the neoplastic sequence of Barrett's oesophagus. Correlation with VEGF expression. *J Pathol* 2000;192:14–18. [PubMed: 10951394]
11. Achen MG, Jeltsch M, Kuk E, Makinen T, Vitali A, Wilks AF, Alitalo K, Stacker SA. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci U S A* 1998;95:548–553. [PubMed: 9435229]
12. Aberle H, Schwartz H, Kemler R. Cadherin-catenin complex: protein interactions and their implications for cadherin function. *J Cell Biochem* 1996;61:514–523. [PubMed: 8806074]
13. Seery JP, Syrigos KN, Karayiannakis AJ, Valizadeh A, Pignatelli M. Abnormal expression of the E-cadherin-catenin complex in dysplastic Barrett's oesophagus. *Acta Oncol* 1999;38:945–948. [PubMed: 10606424]
14. Souza RF, Spechler SJ. Concepts in the prevention of adenocarcinoma of the distal esophagus and proximal stomach. *CA Cancer J Clin* 2005;55:334–351. [PubMed: 16282279]
15. Sullivan PM, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054–1061. [PubMed: 11459866]
16. Reid BJ, Blount PL, Rabinovitch PS. Biomarkers in Barrett's esophagus. *Gastrointest Endosc Clin N Am* 2003;13:369–397. [PubMed: 12916666]
17. Wongsurawat VJ, Finley JC, Galipeau PC, Sanchez CA, Maley CC, Li X, Blount PL, Odze RD, Rabinovitch PS, Reid BJ. Genetic mechanisms of TP53 loss of heterozygosity in Barrett's esophagus: implications for biomarker validation. *Cancer Epidemiol Biomarkers Prev* 2006;15:509–516. [PubMed: 16537709]
18. Rygiel AM, Milano F, Ten Kate FJ, de Groot JG, Peppelenbosch MP, Bergman JJ, Krishnadath KK. Assessment of chromosomal gains as compared to DNA content changes is more useful to detect

- dysplasia in Barrett's esophagus brush cytology specimens. *Genes Chromosomes Cancer* 2008;47:396–404. [PubMed: 18265409]
19. Rabinovitch PS, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 2001;96:3071–3083. [PubMed: 11721752]
 20. Chao DL, Sanchez CA, Galipeau PC, Blount PL, Paulson TG, Cowan DS, Ayub K, Odze RD, Rabinovitch PS, Reid BJ. Cell proliferation, cell cycle abnormalities, and cancer outcome in patients with Barrett's esophagus: a long-term prospective study. *Clin Cancer Res* 2008;14:6988–6995. [PubMed: 18980994]
 21. Reid BJ, Prevo LJ, Galipeau PC, Sanchez CA, Longton G, Levine DS, Blount PL, Rabinovitch PS. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *Am J Gastroenterol* 2001;96:2839–2848. [PubMed: 11693316]
 22. Galipeau PC, Li X, Blount PL, Maley CC, Sanchez CA, Odze RD, Ayub K, Rabinovitch PS, Vaughan TL, Reid BJ. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007;4:e67. [PubMed: 17326708]
 23. Rygiel AM, van Baal JW, Milano F, Wang KK, Ten Kate FJ, Fockens P, Rosmolen WD, Bergman JJ, Peppelenbosch MP, Krishnadath KK. Efficient automated assessment of genetic abnormalities detected by fluorescence in situ hybridization on brush cytology in a Barrett esophagus surveillance population. *Cancer* 2007;109:1980–1988. [PubMed: 17385213]