Part 1 of a 3-Part Series on Genetic Testing: Diseases of the Esophagus And Stomach

Esophagus

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Conventionally, patients are diagnosed as having a medical disorder based on their symptoms; treatment efficacy can vary based on how far the disease has progressed at the point of diagnosis. Genetic testing proposes an alternative approach to diagnosing and managing disease—screening high-risk individuals based on genetic disposition to prevent the onset of symptoms and perhaps the disease altogether. With growing recognition that most chronic diseases reflect complexities with underlying genetic mutations and polymorphisms that target specific organs or systems, genetic testing provides an opportunity to anticipate the development of disease.

Although genetic testing is very accurate, the correlation between genotype and phenotype severity among patients is often variable. There are interacting genes, modifier genes, and environmental cofactors that alter the clinical course and even the susceptibility of individuals with underlying mutations. These genetic and environmental factors will continue to be discovered and placed into context as more individuals with a clear genetic susceptibility to a disease develop a mild or severe clinical course, depending on the influence of these other factors.

**Esophagus**

**Genetic Pathways**

Many genetic and epigenetic alterations may transform a normal or a metaplastic cell into a malignant cell capable of enhanced, independent, and uncontrolled proliferation, prolonged life span, and metastatic potential. Most tumors are characterized by genomic instability that targets 4 classes of genes: proto-oncogenes, tumor-suppressor genes, mismatch repair genes, and mitotic checkpoint genes. Proto-oncogenes are genes that are involved in signal transduction of extracellular stimuli to the cell nucleus or in the regulation of gene expression. Activation of proto-oncogenes by mutation, amplification, or translocation turns them into oncogenes with unregulated activity that enhances proliferation and inhibits programmed cell death (apoptosis). Mutation, deletion or epigenetic silencing and inactivation of tumor-suppressor genes interfere with cell cycle arrest, DNA repair, and apoptosis, thus contributing to tumor formation. Mismatch repair gene deficiency may lead to widespread accumulation of mutations. Finally, inactivation of mitotic checkpoint genes leads to chromosomal instability and abnormal chromosomal number (aneuploidy).

Several key events are involved in the malignant progression of Barrett’s esophagus (BE) and the growth and metastasis of esophageal adenocarcinoma (Table, page 26). Of these, the loss of heterozygosity (LOH) at the 17p chromosomal...
Table. Key Genetic And Molecular Events in BE And Esophageal Cancer

<table>
<thead>
<tr>
<th>Event</th>
<th>Example</th>
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<tbody>
<tr>
<td>Enhanced cell proliferation, increased telomerase activity, and decreased apoptosis</td>
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<tr>
<td>Proto-oncogene mutations</td>
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<td>Tumor-suppressor gene inactivation</td>
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<tr>
<td>Cell cycle events</td>
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<td>Altered cell–cell adhesion</td>
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BE, Barrett’s esophagus

region leads to a nonfunctional p53 protein and altered transcription of p53-regulated genes (p21, bcl-2, and bax-1) that impair DNA repair and apoptosis, thus allowing transmission of damaged DNA and tumor cell growth. Alternatively, activation (eg, mutations) of the genes’ encoding growth factors, their receptors, or the signal transduction genes (ie, ras, myc, src) activate the cell cycle.

Several other genes are involved in cell cycle progression and activation. Cytoskeleton from the G1 to the S phase depends on the activation of the cyclin-dependent kinases CDK4/6 to associate with cyclin D1. This complex, in turn, phosphorylates the retinoblastoma protein (Rb), activating transcription and DNA replication. The p16, p21, and p27 genes inhibit the activation of CDK-cyclin complexes, thus controlling the cell cycle.

Current Status of Genetic Testing

Since the progression of BE to esophageal adenocarcinoma results from several changes in gene structure and expression, genetic tests could help patients in managing their health. Several molecular alterations appear promising as biomarkers for progression and early cancer detection. Among these, alterations in the p53 and p16 genes, cell cycle abnormalities, and aneuploidy are the most well studied.

However, the exact sequence of molecular events is not known, and it probably involves multiple pathways that interact not only with each other, but also with components of gastroesophageal reflux (eg, acid) to enhance malignant transformation. This mechanism has been difficult to study with traditional methods, which are best suited to study 1 gene at a time. However, a newly developed technique, gene expression profiling by DNA microarrays, uses nucleic acid polymers, immobilized on a solid surface, as probes for gene sequences. DNA microarrays are relatively easy to use, yield gene expression measurements for thousands of genes simultaneously, and can be used in a large number of parallel samples. The results can be used to understand what contributes to Barrett’s cell transformation to cancer and the impact of luminal factors (ie, acid and bile) to accurately diagnose and molecularly classify metaplastic and neoplastic tissues, to assess the propensity of these tissues to metastasize, and even to predict their responsiveness to chemotherapy or chemoprevention.

The Future

Ongoing research in the genetics of esophageal carcinogenesis will identify specific genetic abnormalities that will better determine the risk of progression to malignancy and/or the development of cancer prevention strategies. Dysfunction of ras proto-oncogenes, such as c-myc and cyclin D1, are not predictive; in contrast, losses of 4q, 5q, 16q, and 18q are commonly seen in esophageal cancer. Cell–cell adhesion molecules as well as growth factors and their receptors, such as epidermal growth factor receptor (EGFR), c-erb-b2, src, and the cyclooxygenase-2 (COX-2)-induced prostaglandins, appear promising—not only as markers of malignant transformation but also as markers of tumor prognosis.

Over the next several years, gastroenterologists will be able to incorporate use of several biomarkers into their practices. The most promising are those involving the p16 and p53 tumor-suppressor genes, telomerase, and aneuploidy or increased 4N populations (Figures 1 and 2). These markers, used in conjunction with histology, will stratify patients’ risk for Barrett’s adenocarcinoma. Identification of these genetic and cytometric changes may allow clinicians to identify low-risk and patients and lengthen their endoscopic surveillance interval as well as to identify high-risk patients in need of aggressive therapy, such as ablation or esophageal resection.

Stomach

Genetic Susceptibility

Adenocarcinoma of the stomach remains a leading cause of cancer deaths worldwide and continuing to be responsible for the majority of cancer deaths in developing countries. While most cases of gastric cancer appear to occur sporadically, it is estimated that between 8% and 10% of gastric cancer cases are related to an inherited familial component. Familial clustering has been observed in 12% to 25% of gastriac carcinoma cases with dominant inheritance patterns also noted. Case-control studies suggest a small, but consistent increased risk of gastric cancer in first-degree relatives of patients with gastric adenocarcinoma.

Large families with an apparent autosomal dominantly high penetrance inherited predisposition for the development of gastric cancer are rare. In one study, a large Maori kindred manifesting early-onset diffuse gastric cancer was investigated for linkage analysis. The results showed that this disease was linked to the E-cadherin/CDH1 locus on 16q and associated with mutations in this gene. Subsequently, several kindred from a variety of ethnic backgrounds manifesting a diffuse, poorly differenti-ated gastric cancer predisposition trait have been shown to harbor germline E-cadherin alterations that cosegregate with these cancers. Thus, E-cadherin mutation testing should be considered in the appropriate clinical setting (Figure 3).

A now well-characterized inherited trait that predisposes a patient to gastric cancer is hereditary non-polyposis colorectal cancer (HNPCC). Germline genetic abnormalities of mismatch repair genes underlying this disease entity have recently been unveiled, and it includes potential tumor development in a variety of tissue types. In a Finnish HNPCC registry study, gastric carcinomas that occurred were predominantly of the intestinal type, without Helicobacter pylori infection detected, were diagnosed at the mean age of 56 years, and most exhibited microsatellite instability (MSI).

There also has been a report of gastric carcinoma in an extended Li-Fraumeni syndrome kindred with an underlying p53 germline alteration. Gastric cancers have also been noted to occur in patients with gastrointestinal polyposis disease entities such as familial adenomatous polyposis (FAP) and Peutz-Jeghers syndrome. Interestingly, an increased risk of gastric cancer associated with FAP has been reported in populations at high risk for gastric cancer, such as Asians, whereas no increased risk was exhibited in other populations. Overall, gastric
carcinoma is rare, and the exact contribution of the polyposis and underlying germline alterations of APC and LKB1/STK11 to gastric adenocarcinoma development is unclear. Finally, rare kindreds exhibiting site-specific gastric cancer predilections have been reported, occasionally associated with other inherited abnormalities.\textsuperscript{24,25}

The most common mesenchymal tumors of the GI tract are gastrointestinal stromal tumors (GIST), one subset of which is commonly solitary with an activating gain-of-function mutation of the c-KIT proto-oncogene.\textsuperscript{26} A germline mutation of c-KIT between the transmembrane and tyrosine kinase domains similar to the somatic changes previously noted in sporadic cases was also demonstrated in a family manifesting multiple GISTS that cosegregated with disease.\textsuperscript{27} The interstitial cells of Cajal that give rise to some of these tumors express KIT as well as CD34. The new relatively specific tyrosine kinase inhibitor, imatinib mesylate (Gleevec, Novartis), affects KIT and has been demonstrated to be effective in the treatment of GISTs.\textsuperscript{28}

**Molecular Genetics**

Most cases of stomach cancer are sporadic in nature, with rare reports of inherited gastric cancer predisposition traits. Cytogenetic studies have been unrevealing in identifying consistent, overt chromosomal aberrations in gastric cancers. LOH studies and comparative genomic hybridization analyses have identified several loci with significant allelic loss, thus indicating the possibility of harboring a tumor-suppressor gene important in gastric tumorigenesis.\textsuperscript{29,30} The exact target(s) of loss or gain in most of these chromosomal regions, including 4, 5q, 9p, 17p, 18q, and 20q, remains to be clarified.

Multiple somatic alterations have been described in gastric carcinomas at the molecular level. The significance of these changes in gastric tumorigenesis remains to be established in most instances. Characterization of critical molecular alterations will ultimately provide new avenues to combat these cancers.

The p53 gene is consistently altered in a majority of gastric cancer cases.\textsuperscript{31} Many studies have used immunohistochemical analysis of tumors in an effort to detect excessive expression of p53 as an indirect means of identifying mutations of this gene, but this assay does not appear to have consistent prognostic value in patients with gastric cancers.\textsuperscript{32,33}

A high degree of MSI (MSI-H) and associated alteration of the transforming growth factor–beta receptor type II (TGFB-II), IGFIR, BAX, E2F-4, hMSH2, and hMSH6 genes are found in a subset of gastric carcinomas.\textsuperscript{34,35} Although the majority of gastric cancers exhibit significant aneuploidy, MSH6 has been found in a subset of sporadic gastric carcinomas ranging from 13% to 44%.\textsuperscript{32} Abnormal loss of protein expression of either hMLH1 or hMSH2 was demonstrated in all cases exhibiting MSI-H.\textsuperscript{35} Altered expression of hMLH1 was associated with an abnormal form of the promoter region of hMLH1 in MSH-H cases, suggesting a silencing role of hypermethylation.\textsuperscript{36,37} The degree of genome-wide instability also varies with more significant instability (eg, MSH-H >53%) abnormal loci) occurring in only 16% of gastric cancers, usually of the subcardial intestinal or mixed type, and those with less frequent lymph node or vessel invasion, prominent lymphoid infiltration, and better prognosis.\textsuperscript{38}

Amplification and overexpression of the c-met gene, which encodes a tyrosine kinase receptor for the hepatocyte growth factor, has been reported in gastric carcinomas.\textsuperscript{39} The epidermal growth factor (EGF) is expressed in approximately a quarter of gastric cancers, and the alteration of fibroblast growth factor receptor (FGFR) and Kras is observed in a number of cases.\textsuperscript{40} Telomerase activity has been detected by a polymerase chain reaction (PCR)-based assay frequently in the late stages of gastric tumors and observed to be associated with a poor prognosis.\textsuperscript{41} Amplification of c-erb-b2 has been demonstrated in a small subset (approximately 10%) of gastric cancers and overexpression observed to be associated with a poor prognosis.\textsuperscript{42} Membrane-type matrix metalloproteinase (MMP) was preferentially expressed in some gastric cancer cells with colocalization and activation of the zymogen, proMMP.\textsuperscript{43} Increased plasminogen activation has been reported, as well in several gastric tumors. Cell cycle regulator alterations such as a loss of p27 has been observed to correlate with advanced disease. Cell adhesion molecule abnormalities such as those involving E-cadherin may play an important role in sporadic diffuse-type gastric cancer development, as well. Specific alterations such as these need true prevalence determination and further characterization of significant changes in gastric tumors before genetic tests can be designed for clinical utility and validation.

Figure 3. Algorithm for guidance in managing familial GC kindreds.
Conclusion

Genetic testing is one of the most powerful and important developments in battling GI diseases. This area will continue to develop over the coming decades and is likely to revolutionize medicine. As with any new technology, there are risks, benefits, and limitations. Although these tests are highly accurate, the major concern is insurance discrimination.55,56 Control of this information is very important because it has lifelong implications for the patient and may have implications for family members, as well. A national debate is under way on how to protect individuals undergoing genetic testing, while giving insurance companies appropriate information on disease risk. Growing interest among patients for anonymous testing has also emerged because patients want to know about their own health and often, that of their children without risking higher premiums or denial of health insurance coverage.50 The benefit of genetic testing is that these simple tests are extremely accurate and have strong prognostic implications. These tests do not need to be repeated, but remain relevant over a lifetime.

These and other new important genetic tests are available to practicing gastroenterologists. It is clear that a careful educational program is needed in order to help physicians know when to order specific tests, how to interpret the results, and how to counsel the patients. Based on the power of these tests, genetics will be developing rapidly, with panels of genomic markers and biological tests soon becoming available. It will also be the responsibility of academic institutions and the medical industry to provide a clear understanding of the disease process and guidelines for the use of these tests.

As we learn more about the human genome, we find more information that may be pertinent to GI diseases of the esophagus and stomach. The numerous discoveries of the human genome that are pertinent to these GI diseases are continually evolving as translational research makes great strides in the areas of biomarkers, genetic diagnosis, and genetic therapies. Yet with extensive information about genetic implications and its implications on disease management, it can be difficult to determine what is clinically important for the gastroenterologist today.

In this rapidly progressing genetic evolution, it is important that the gastroenterologist become familiar with the current roles of genetics in managing upper GI diseases in order to provide the best patient care possible. Since more discoveries are being made in genetics every day, continuing education on the clinical relevance of the genetic discoveries is one important way to decipher the new genetic code.

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