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Biomarkers and Molecular Diagnostic Tests in Gastrointestinal Tract and Pancreatic Neoplasms

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Abstract

Standard practice for the diagnosis of neoplasms in the gastrointestinal tract is based on histopathologic diagnosis in combination with clinical information. With the completion of the Human Genome Project in the past few years, our understanding of the contribution of the genome to human disease has sky rocketed. This has led to the incorporation of knowledge about genes involved in disease processes into clinical decision making. However, the rate of movement of molecular biomarkers into widespread clinical application has lagged behind that of biomarker discovery. Nevertheless, there are a number of molecular biomarkers available for use in the diagnosis and management of gastrointestinal tract neoplasms. This article reviews the more common molecular biomarkers currently available for luminal gastrointestinal tract and pancreatic neoplasms. For neoplasms of the esophagus in which no biomarkers are currently incorporated into routine clinical practice, the biomarkers that have shown the most promise in early clinical validation studies are discussed.

Introduction

The completion of the human genome project in 2003 has accelerated the pace of discovery of genes involved in the genesis of human disease. [1] From these discoveries, the concepts of “genomic medicine” and “personalized medicine” have emerged in which genomic tests are incorporated into the clinical management of various disease states. [1] The area in which genomic medicine is most readily being used is in the diagnosis and therapy of human tumors including those of the gastrointestinal tract. Standard practice for the diagnosis of neoplasms in the gastrointestinal tract is the histopathologic finding of dysplasia and/or cancer. While the histopathologic diagnosis of cancer of the gastrointestinal tract is generally unambiguous, there are a number of limitations in diagnosing dysplasia including biopsy sampling error, inter-observer variability, and difficulty in obtaining adequate biopsy

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specimens from a number of gastrointestinal organs. [2] Thus in the era of genomic medicine, molecular pathology and the use of "biomarkers" has become a rapidly expanding field playing a central role not only in the diagnosis of neoplasia, but also in the selection of "tailored" anti-cancer therapies. [3]

The Biomarker Definitions Working Group of the National Institutes of Health Director's Initiative on Biomarkers and Surrogate Endpoints define a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." [4] Clinically, biomarkers can be used for (1) diagnosis which identifies those patients with a disease or abnormal condition, (2) prognosis, (3) progression which includes the staging and extent of disease, and (4) therapeutic response to an intervention. [4] In general, molecular biomarkers fall into broad categories associated with biologic properties of malignant cells that must be acquired by benign cells as cancer arises. These properties include cell division independent of exogenous mitogenic stimulation, resistance to growth-inhibitory signals, avoidance of apoptosis, limitless replication, development of adequate vascular supplies, the capacity to invade and metastasize, and the establishment of an inflammatory microenvironment. [5,6] This article reviews the more common clinically available molecular biomarkers for neoplasms of the stomach, pancreas, and colon. For cancers of the esophagus there are no biomarkers currently being incorporated into routine clinical practice, thus the biomarkers that have shown the most promise in clinical validation studies are discussed.

Esophagus

Cancers of the esophagus are among the most deadly of gastrointestinal malignancies with 5 year mortality rates exceeding 87%. [7] Squamous cell carcinoma and adenocarcinoma are the two major histologic types of esophageal cancer. Worldwide, more than 90% of esophageal cancers are squamous cell carcinoma; however, in the US there has been a decline in the incidence of of squamous cell carcinoma paralleled by a dramatic rise in the incidence of adenocarcinoma. [8] Survival for both tumor types can be improved if they are detected in early stages.[9,10] Currently, there are no biomarkers in widespread clinical use for either histologic type of esophageal cancer, however the biomarkers that have shown the most promise in early clinical validation studies are discussed below.

Esophageal Squamous Cell Carcinoma—Currently, esophageal balloon cytology is one of the techniques used in screening for esophageal squamous cell carcinoma.[11] Unfortunately, the sensitivity of this technique for detecting squamous dysplasia or early cancer has been shown to be only approximately 50%.[12] In order to improve the detection rates for squamous dysplasia and early cancer, a DNA methylation-based biomarker gene panel has been used on specimens collected by esophageal balloon cytology. DNA methylation is a mechanism whereby cells can turn off gene expression by the addition of methyl groups to nucleotides in the promoter regions of genes. However even when the "best" DNA methylation-based biomarker gene panel was used on specimens collected by esophageal balloon cytology, the sensitivity was only 50% for the detection of squamous cell dysplasia. [12] Additional studies on molecular biomarkers for early detection of esophageal squamous carcinoma are clearly warranted.

Esophageal Adenocarcinoma—The large majority of esophageal adenocarcinomas are thought to arise from the metaplastic epithelium of Barrett's esophagus. [13] Periodic endoscopic surveillance with biopsies to detect dysplasia is the current practice to reduce the risk of developing invasive carcinoma in patients with Barrett's esophagus. [13–15] Since Barrett's esophagus is a major risk factor for esophageal adenocarcinoma, molecular biomarkers capable of identifying those patients with Barrett's esophagus who have the

highest risk for cancer progression have been the primary focus of research efforts in this area. Table 1 highlights the biomarkers that have advanced the farthest for use as predictors of disease progression in patients with Barrett's esophagus. Very little has been done to uncover molecular biomarkers indicative of the presence of Barrett's esophagus itself. [16,17]

Among the more promising biomarkers predictive of disease progression for patients with Barrett's esophagus is the use of aneuploidy/increased tetraploidy. Aneuploidy refers to an abnormal number of chromosomes. Normal cells contain two copies of each chromosome (diploid) which increase to four copies (tetraploid) just prior to cell division. Several studies have shown that aneuploidy and/or increased tetraploidy (4n fractions present in over 6% of cells within a tissue), as detected by flow cytometry, may be better predictors of neoplastic progression than the histologic diagnosis of no dysplasia, indefinite, or low grade dysplasia. [18,19]

Another promising biomarker to predict cancer progression in Barrett's esophagus is 17pLOH. Loss of heterozygosity (LOH) refers to the loss of a section of a chromosome containing one of the two alleles for a gene. The tumor suppressor gene *TP53* (p53) is located on the small arm (p) of chromosome 17 and is thought to be one of the genes targeted by the allelic loss. In one study, 17pLOH in biopsy specimens from patients with Barrett's esophagus was associated with a 3 year cumulative incidence of cancer of 38% regardless of the presence or absence of dysplasia. [20] In comparison, biopsy specimens in which both alleles of 17p were retained had a cumulative incidence of cancer at 3 years of 3.3%. [20] While results such as these are promising, the use of flow cytometry is technically difficult and is currently available only in highly specialized research laboratories. Alternative techniques such as automated image cytometry and FISH, which are more feasible for routine clinical practice, are currently being explored.[21,22]

The complexity of human tumorigenesis makes it unlikely that a single molecular biomarker will be adequate to predict cancer formation. Recent studies have begun to investigate panels of biomarkers for use in Barrett's esophagus. For example, a combination panel of aneuploidy/increased tetraploidy, 17pLOH, and 9pLOH (the chromosomal locus for *p16*) has been investigated in patients with Barrett's esophagus. [23] The use of a DNA methylation-based biomarker panel (containing 6 genes) on biopsies from metaplastic Barrett's epithelium, in combination with the patient's age, and the length of Barrett's segment has been used to generate a "prediction model" for neoplastic progression. [24]

Stomach

Gastric Adenocarcinoma—The two major histological variants of adenocarcinoma of the stomach are diffuse-type and intestinal-type gastric cancers. In general, individuals with diffuse-type tumors typically have a worse prognosis than those with intestinal-type tumors. *ERBB2* is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. Amplification of the *ERBB2* gene and/or overexpression of HER2, the protein product of *ERBB2*, have been identified in breast, ovarian, esophageal adenocarcinoma, and gastric cancers, amongst others.[25,26] HER2 is more commonly overexpressed in intestinal type (21.5%) than in the diffuse type (2%) gastric adenocarcinoma.[26] HER2 expression in gastric cancers has been proposed as a potential biomarker to predict prognosis and therapeutic response. Earlier studies suggested that HER2-positive gastric cancer had a poor prognosis, however more recent data suggest that HER2 expression may have no prognostic relevance [26,27]. Trastuzumab is a monoclonal antibody which binds to and inhibits HER2 signaling. Trastuzumab in addition to standard chemotherapeutic agents has shown a benefit in patients with HER-2 positive gastric cancers, suggesting that HER2 can be used as a biomarker predictive of therapeutic response

(Table 2). [28] Immunohistochemistry and FISH are the most common methodologies currently available for assessing HER2 status. [25,29–31]

The current model for development of gastric adenocarcinoma of the intestinal type is a step wise progression from non-atrophic gastritis, multifocal atrophic gastritis, intestinal metaplasia, dysplasia, and finally adenocarcinoma. [32] In high risk populations for intestinal-type gastric adenocarcinoma, such as the Japanese, measurements of serum pepsinogen levels have been shown to be useful biomarkers for the diagnosis of severe gastric atrophy [with or without intestinal metaplasia (Table 2)]. Pateints with extensive atrophy are at high risk of neoplastic progression.[33] Pepsinogen I (PGI) and II (PGII) are produced by chief cells and mucous neck cells in the oxyntic mucosa. While PGI is produced exclusively by these cells, PGII is also produced by cells in the pyloric and Brunner's glands. [32] When atrophy affect the gastric corpus (with a loss of the PGI-producing cell mass), there is a marked decrease in the levels of PGI and a low serum ratio of PGI to PGII (which is unaffected because PGII is still being produced by cells in the pyloric and Brunner's glands). [32] In several Japanese and some European studies, serum PGI levels <70 ng/l and a PGI/PGII ratio < 3.0 were found to have a high sensitivity (approximately70%) and almost perfect specificity (approximately 97%) for the detection of gastric corpus atrophy. [34] Patients identified by abnormal pepsinogen ratio can then be referred for endoscopic evaluation.

Hereditary Diffuse Gastric Cancer—While most cases of gastric cancer are sporadic, familial clustering is observed in approximately 10% of cases.[35] Hereditary diffuse gastric cancer (HDGC) is characterized by an autosomal dominant susceptibility for diffuse-type gastric adenocarcinoma which accounts for about 1–3% of the familial cases. [35] The majority of patients with HDGC carry mutations in the tumor suppressor gene *CDH1*, also known as E-cadherin. *CDH1* mutations have been described in diffuse-type, but not intestinal-type gastric cancer.[36–38] In 2004, a revision of the criteria for consideration of *CDH1* molecular genetic testing in patients with suspected HDGC was proposed. Table 3 lists the revised criteria which are applicable to individuals with gastric cancer in North America and other regions of low gastric cancer incidence; however, they may not be appropriate for use in high-incidence regions such as Japan and Korea. [39] Sequencing of the coding region of *CDH1*, as well as deletion/duplication analysis, is available for clinical testing (Table 2). Molecular testing for *CDH1* mutations has a detection rate of about 30% in patients who meet clinical criteria for a diagnosis of HDGC in the absence of a known familial disease-causing mutation. Since current data suggest that a prophylactic total gastrectomy should be considered in asymptomatic CDH1 mutation carriers, mutation testing for HDGC should never be performed without the support of appropriate genetic and clinical counseling. [40]

Gastrointestinal stromal tumor—Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the GI tract. While GISTs occur most often in the stomach, they can be found elsewhere the luminal gut and exceptionally also outside the digestive system. The cell of origin has been identified as the interstitial cell of Cajal, which regulates peristalsis in the digestive tract. [41] Most GISTs are sporadic, arising because a somatic gain-of-function mutation in the tyrosine kinase gene *KIT*. [42] Mutations in *KIT* result in an oncogene with ligand-independent tyrosine kinase activity. The other genetic mutation identified in 3–5% of GISTs occurs in the *PDGFRA* gene (which appears to be mutually exclusive of *KIT* mutations). [43] Imatinib, a tyrosine kinase inhibitor used for the treatment of Philadelphia-positive chronic myelogenous leukemia, has shown benefit in the treatment of GISTs because the KIT and PDGFRA kinases are targets of this chemotherapeutic agent. [44] Localized GISTs, in which surgical resection is complete, do not require further medical treatment. However, patients with unresectable tumors, advanced,

metastatic, or recurrent disease may be candidates for Imatinib treatment. More recent studies suggest there may be a role for adjuvant imatinib therapy after resection of the primary GIST to increase rates of recurrence-free survival. [45]

Since most GISTs respond to targeted therapy with imatinib, it is essential to differentiate GISTs from other tumors considered in the differential diagnosis. KIT is expressed in 95% of GISTs, and is therefore a major diagnostic criterion for GISTs. [46] Another recently identified diagnostic marker now used in some laboratories is the immunostain for DOG1 (*ANO1)*. [47,48] Expression of the KIT and DOG1 proteins can be used as diagnostic biomarkers (Table 2). In addition, mutations in the *KIT* and *PDGFRA* genes can be used as biomarkers to aid in predicting a therapeutic response to imatinib therapy; *KIT* mutations can also be used to predict overall prognosis (Table 2). For example, GIST patients with *KIT* exon 11 mutations show a significantly higher partial response rate to imatinib (83.5%) than those with *KIT* exon 9 mutations, no mutations, or mutations in *PDGFRA* (47.8%). [49] Furthermore, patients whose GISTs harbored *KIT* exon 11 mutations also had a longer event-free and overall survival than those whose tumors contained *KIT* exon 9 mutations, wild-type *KIT*, or wild-type *PDGFRA*.[49] Additionally, patients with exon 9 mutations in *KIT* treated with imatinib had a relative risk of disease progression of 171% and a relative risk of death of 190% compared to those treated patients with exon 11 *KIT* mutations. [50] In patients with exon 9 *KIT* mutations, improved progression-free survival could be achieved with a higher dose of imatinib (800mg vs. 400 mg/day). [50] Despite the initial response of most GISTs to imatinib, data suggest that the tumors eventually become resistant to this therapy due to the acquisition of secondary mutations within the *KIT* gene. [51] Thus additional agents that target the KIT and PDGFRA kinases are currently under investigation. [52] Since mutations in the *KIT* gene are biomarkers of therapeutic response to imatinib, mutation analyses should be pursued regardless of whether the tumor is KIT (CD117) positive or negative by immunohistochemistry. A list of laboratories offering research and clinical mutation analyses for *KIT* and *PDGFRA* can be found at http://www.amptestdirectory.org.

Pancreas

Pancreatic Adenocarcinoma—Pancreatic adenocarcinomas originate from the ductal epithelium and progress from minimal dysplasia [pancreatic intraepithelial neoplasia (PIN) grades 1A and 1B) to severe dysplasia [PIN grades 2 and 3) before becoming an invasive tumor.[53] No biomarkers are currently recommended for routine use to screen for pancreatic cancer. In contrast, CA19-9 has been shown to be a clinically useful biomarker for monitoring therapeutic response and for the early detection of recurrent disease after treatment (Table 4). [54–57] However, CA19-9 is not without its limitations including the lack of specificity for pancreatic cancer since other conditions such as biliary cholestasis can elevate the levels of this protein. [53] While most cases of pancreatic cancer are sporadic, there is familial clustering observed in approximately 5–10% of cases and in 10–20% of such cases a hereditary component may be involved. [58] There are a number of heritable conditions that increase the risk of an individual to develop pancreatic cancer, and specific genetic tests are available to identify causative mutations for most of these heritable conditions. [58] However, no wide-spread clinical recommendations are available at this time for pancreatic cancer screening in these high risk individuals.[58]

Pancreatic Cystic Neoplasms—Mucinous cystadenomas and intraductal papillary mucinous neoplasia are two types of pancreatic cystic neoplasms that carry a risk of progressing to malignancy. Because of this increased risk, surgical resection of these lesions should be considered. [59,60] In contrast, small, benign serous cystadenomas can be followed conservatively. [61,62] Therefore, distinguishing premalignant mucinous cysts from benign non-mucinous cystic lesions is of clinical relevance. Endoscopic ultrasound (EUS) has the

ability to detect cystic lesions of the pancreas with a high degree of safety, but is not sufficient to distinguish non-mucinous from mucinous cystic lesions.[62] However, EUS does allow for the aspiration of the cyst fluid which can then be sent for several types of analyses including cytology, CEA level, and molecular diagnostics. One consideration in ordering tests on aspirated cyst fluid is the volume available for diagnostic testing. Prioritization of testing should be considered when cyst fluid aspirates are of low volume. Since, histopathology is an essential tool in the diagnosis and classification of mucinous pancreatic cysts; aspirate fluid cytology should be ordered first. [63] Any remaining specimen can be considered for ancillary testing (CEA level, molecular diagnostics) as discussed below. Communication with the laboratory is essential, and protocols should be in place to ensure proper allocation of cyst fluid, especially when the volume is low.

Currently, the detection of CEA levels in pancreatic cyst fluid is the most accurate diagnostic biomarker to distinguish non-mucinous from mucinous cyts (Table 4). [60,62] It is important to keep in mind however that CEA levels do not distinguish whether the mucinous cyst is benign or malignant.[60,62] Data suggest that a cyst fluid CEA level of <192 ng/ml is indicative of a non-mucinous cyst whereas CEA levels >192–200 ng/ml are suggestive of a mucinous cyst. [62] Although pancreatic cyst fluid CEA is most accurate biomarker available, its detection is fraught with limitations that must be kept in mind when deciding whether to send cyst fluid for CEA analysis. Commercial assays for CEA determinations have not been validated in cyst fluid contents and the techniques for handling and processing the cyst fluid for CEA measurements are not standardized. [62] Thus the cut-off value of a CEA level of 192 in cyst fluid to distinguish non-mucinous from mucinous cysts derived by the laboratory at the Massachusetts General Hospital may not be the appropriate cut-off value when the test is performed at another laboratory. [62] In addition, measurement of CEA levels in cyst fluid is not approved by the FDA and is considered an “off-label” use. [62]

Molecular testing of cyst fluid has increased in recent years due to the commercial availability of testing [PathFinderTG, RedPath Integrated Pathology, Pittsburgh, PA (Table 4)] and the ability of the test to be done on extremely small amounts of cyst fluid (200 μl).[62] In the PANDA study, Khalid *et al.* investigated pancreatic cyst fluid DNA in 113 patients using a proprietary DNA analysis method (which is now the commercially available PathFinderTG test).[64] Included in the study were 40 malignant, 48 premalignant and 25 benign cysts. The molecular analysis consisted of sequencing of the first exon of *KRAS* to identify a point mutation, loss of heterozygosity (LOH) in a panel 15 of tumor suppressor genes, and determination of DNA quantity and quantity. The most specific test for detecting mucinous cysts in this cohort was the presence of a *KRAS* mutation in exon 1 (96% specificity), however the sensitivity was low (45%). The most accurate test for diagnosing malignant cysts was an allelic loss amplitude >80%, which was determined by calculating the proportion of cellular DNA demonstrating LOH.[64]

Another study by Shen *et al.* investigated the same proprietary molecular tests panel, comparing the panel to a clinical consensus diagnosis of pancreatic cysts. [65] The clinical consensus diagnosed pancreatic cysts as malignant, benign mucinous, or benign non-mucinous using histology or a combination of 2 of the following 3 characteristics: 1) endoscopic ultrasound findings, 2) CEA measurement from the cyst fluid, or 3) cytologic findings. There was concordance between consensus clinical diagnosis and molecular diagnosis in 5 of 6 (83%) malignant cysts; 13 of 15 (87%) benign mucinous cysts; and 13 of 14 (93%) benign non-mucinous cysts. By molecular diagnosis, there was one false-negative diagnosis of a malignant cyst, two benign mucinous cysts classified as benign non-mucinous, and one non-mucinous cyst called a benign mucinous cyst. [65] While these data are promising for the use of molecular diagnostics to distinguish non-mucinous from mucinous cysts and malignant from benign cysts, larger prospective randomized controlled

clinical trials are still needed to determine the clinical utility of pancreatic fluid molecular testing.

Colon

Sporadic colorectal cancer—Worldwide colorectal cancer incidence and mortality rates are on the rise, in contrast, they have declined in the United States perhaps because of the advances in colorectal cancer screening. [66,67] In recent years, stool DNA tests have been the molecular approach to colorectal cancer screening that has been most intensively studied.[68] Several studies have compared the rates of detection of advanced adenomas and invasive tumors between guaiac testing and stool DNA tests. One study compared fecal occult blood testing using Hemoccult and HemoccultSensa (Beckman Coulter, Fulterton, CA), a stool DNA test with a 23-marker assay (SDT-1) and another novel stool DNA test (SDT-2) with only 3 targeted markers; colonoscopy was used as the "gold standard" for adenomas or cancer detection. [69] Overall, there was no significant difference in rates of detection for colorectal neoplasia between the HemoccultSensa and the SDT-1; detection rates for SDT-1 were significantly greater than Hemoccult. [69] Detection rates for colorectal neoplasia by SDT-2 were significantly better than either of the fecal occult blood detection assays. [69] Moreover, SDT-2 had significantly higher rates of detection for advanced adenomas < 1 cm compared to either Hemoccult or HemoccultSensa. [69] One drawback, however, was a higher false positive rate (16%) using SDT-2 (i.e. colonoscopy was normal in those with a positive test result) than either Hemoccult (4%) or HemoccultSensa (5%).[69] Nevertheless, stool DNA testing as a screening modality (Table 5) has recently been endorsed by the American Cancer Society, the US Multi-Society Task Force, the American College of Radiology, and the American College of Gastroenterology, but not yet by the US Preventive Services Task Force. [70,71]

Clinical and pathologic staging are currently the gold standard methods of determining the prognosis in patients with colorectal cancer. A major limitation of conventional staging is that it does not address whether and how patients will respond to chemotherapy. In the past few years *KRAS* has become a biomarker of interest with respect to therapeutic response to anti-EGFR therapies (Table 5). Cetuximab and panitumumab are anti-EGFR monoclonal antibodies which have been approved by the FDA for treatment of patients with metastatic colorectal cancer. [72] EGFR receptor is expressed in approximately 85% of patients with metastatic colorectal cancer, however only a proportion of patients demonstrate a clinical benefit from treatment. [73] Mutations of *KRAS* which activate signaling have been found to confer resistance to anti-EGFR monoclonal antibody therapies.[73] In fact, the National Comprehensive Cancer Network and American Society of Clinical Oncology guidelines recommend that all metastatic colorectal tumors should be tested for *KRAS* mutations before beginning anti-EGFR monoclonal antibody therapy and only in those patients whose tumors contain wild-type *KRAS* should therapy be initiated. [73–75] In general, mutations in *KRAS* have been found in 30–40% of all colorectal cancers. [73]

KRAS mutation testing is performed on DNA extracted most commonly from formalin-fixed, paraffin embedded tumor.[73] Factors that can impact the quality the *KRAS* testing include the purity and quantity of tumor cells and the fixation and processing of the tissues therefore pathologist expertise is essential. There are a variety of techniques that can be used for mutation analysis of *KRAS* including real-time polymerase chain reaction, Sanger sequencing, or pyrosequencing. [76]

Familial adenomatous polyposis—Familial adenomatous polyposis (FAP) is an autosomal dominant colon cancer predisposition syndrome, caused by mutations in the *APC* tumor suppressor gene. Patients with FAP develop hundreds to thousands of adenomatous

colonic polyps which, if untreated, invariably lead to colon cancer. The clinical diagnosis of FAP is made when a patients has ≥ 100 colorectal adenomas (usually occurring in adolescence or early adulthood). [77] A milder form of FAP (attenuated FAP) is characterized by <100 colon adenomatous polyps and a later onset of disease. [78] FAP is most commonly caused by nonsense or frameshift mutations in *APC* which lead to premature protein truncation and loss of APC function. Full gene sequencing is currently the gold standard for detecting *APC* mutations (Table 5). [79] In addition, deletion/duplication analysis and protein truncation assays are also clinically available tests. Although clinicians can directly order the genetic test for FAP, the 2008 American College of Gastroenterology Guidelines for Colorectal Cancer Screening suggests that patients with suspected FAP should be referred first to a genetic counselor and should be informed of the protections endowed by current laws in regards to life insurance, long-term care insurance and disability insurance. [71]

MUTYH-associated adenomatous polyposis—MUTYH-associated adenomatous polyposis can resemble FAP or attenuated FAP. In 2002, Al-Tassan et al. reported finding mutations in both alleles of the *MUTYH* gene in a British family with recessive inheritance of colorectal adenomas and cancer. [80] Subsequent studies have since confirmed the role of bi-allelic MUTYH mutations in patients with >10 adenomas. [80–82] Thus, if APC mutation is negative in individuals suspected of having FAP, then testing for *MYH* mutation should be done keeping in mind that appropriate genetic counseling should be done beforehand (Table 5). [71]

Hereditary non-polyposis colorectal cancer—Hereditary non-polyposis colorectal cancer (HNPCC; also known as Lynch Syndrome) is autosomal dominant inherited predisposition to colorectal and other cancers (e.g. brain, endometrium, hepatobiliary tract, ovary, pancreas, skin, stomach, small intestine, and upper urinary tract) as a result of a germline mutation in DNA mismatch repair (MMR) genes (*MLH1, MSH2, MSH6, PMS2,* and possibly *MLH3*).[83] Faulty DNA mismatch repair leads to an accumulation of genetic mutations, which can ultimately result in malignant transformation of cells. Germline mutations in *MLH1* and *MSH2* account for the vast majority of mutations in HNPCC families (approximately 90%). Mutations in the other mismatch repair genes are less common; *MSH6* in approximately 7%–10%, and *PMS2* fewer than 5% of mutations in families with HNPCC. [84]

Small repetitive sequences of nucleotides located throughout the genome called microsatellites, are particularly prone to acquiring mutations in the setting of defective mismatch repair. The term “microsatellite instability” (MSI) has been used to describe tumors with these mutated short sequences of DNA. A validated panel of five microsatellite loci is used to determine whether a tumor demonstrates MSI. [85] Tumor DNA demonstrating abnormalities in two or more of the microsatellite loci are classified as MSI-high (MSI-H). [83] In patients who meet the Bethesda criteria for HNPCC, available tumor tissue should be initially tested for MSI. Because only a fraction of individuals who meet the clinical criteria in the Bethesda Guidelines will in fact have HNPCC, germline mutational testing has a diagnostic yield of <50%. [83] The addition of MSI testing on tumor tissue adds to the predictive ability of the clinical criteria and it is only those patients whose tumors demonstrate MSI-H who are advised to undergo genetic testing (Table 5). [83] DNA testing consists of mutation scanning and full gene sequence analysis of the MMR genes (Table 5). Deletion/duplication analysis is used to identifying large deletions or gene rearrangements not detected by sequencing. Promoter methylation has been identified as another mechanism of loss of *MLH1* function. Methylation-specific PCR can be performed to identify this epigenetic alteration. However, HNPCC is not the only setting in which MSI can be identified. For example, 10 to 20% of sporadic colorectal adenocarcinomas of the colon will

demonstrate MSI due to *MLH1* promoter methylation or acquired somatic mutations in a mismatch repair genes. [86]

Although a finding of MSI-H is quite sensitive for HNPCC, there are a small proportion of patients with mutations in MMR genes that will not be detected by MSI testing. Therefore, immunohistochemistry for MSH2, MSH6, MLH1, and PMS2 expression can be done to supplement the MSI testing. [83] However, some gene mutations may result in loss of gene function, and not necessarily in loss of the entire protein product. Therefore, there is a risk of a false-positive immunostain (i.e. the protein is detected by immunohistochemistry but it is not functionally active).[87] From a practical standpoint, immunohistochemistry may a better choice for first round of testing because it is more available than molecular testing for MSI. Regardless of whether immunohistochemistry or MSI testing is done initially, DNA testing remains the gold standard for confirming a mutation in a mismatch repair gene. Once a specific mutation has been identified in an affected family, targeted molecular analysis can be ordered in relatives, saving both time and costs. As noted above for genetic testing in suspected FAP patients, the involvement of a genetic counselor prior to genetic testing for HNPCC is also suggested. [83]

In regards to these molecular biomarkers predicting response to therapy, patients with stage II or stage III HNPCC colorectal tumors or sporadic colorectal tumors demonstrating *MLH1* silencing by methylation have been found to derive either no benefit or a 2–3 fold increase in mortality by adjuvant 5-FU-based chemotherapy (Table 5). [88–93] Therefore, 5-FU-based adjuvant chemotherapy regimens should not be offered to these patients.

Conclusion

Completion of the human genome project has ushered in an era of genomic medicine. Advances in understanding the involvement of specific genes in human disease has led to improvements in diagnosis and management. As a result of this wave of biomedical discovery, there are a number of molecular biomarkers available for clinical use that can aid in diagnosis and therapeutic decision making for gastrointestinal cancers. In addition, multiple tools available on the web for assistance with questions or decisions regarding molecular testing. The Association for Molecular Pathology has a test directory (http://www.amptestdirectory.org/) where laboratories voluntarily list their research or clinical molecular tests. Furthermore, GeneTests, a publicly funded medical genetics information resource developed for physicians, other healthcare providers, and researchers, houses resources about inherited genetic disorders. As part of the GeneTests web site are the GeneReviews. These are disease-specific reviews written by international experts who cover the role of genetic testing and its application to disease management. The GeneTests website also includes a national and international directory of genetic testing laboratories as well as genetic counseling clinics, and prenatal diagnostic clinics. The efforts devoted to genomic discoveries have changed our understanding of disease pathogenesis over the past few years and it is anticipated that such knowledge will “translate” into further applications for molecular pathology in the management of diseases of the gastrointestinal tract.

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Table 1

More promising molecular biomarkers for esophageal neoplasia. These potential biomarkers are currently under investigation.

Tumor	Biomarker	Category
Squamous cell carcinoma	*None*	
Adenocarcinoma	aneuploidy/increased tetraploidy	Progression
	17pLOH (*p53*)	Progression
	panel of aneuploidy/increased tetraploidy, 17pLOH, & 9pLOH (*p16*)	Progression
	Methylation-based gene panel	Progression

Table 2

Biomarkers for gastric neoplasia

Tumor	Biomarker	Category
Gastric Adenocarcinoma	HER2 (*ERBB2* gene)	Therapeutic Response
	Serum pepsinogensI and II	Diagnosis
Hereditary Diffuse Gastric Cancer	*CDH1* gene	Diagnosis
Gastrointestinal stromal tumor(GIST)	CD117 (KITprotein)	Diagnosis
	DOG1	Diagnosis
	KIT gene	Therapeutic Response; Prognosis
	PDGFRA gene	Therapeutic Response

Table 3

Clinical Criteria for CDH1 Mutation Testing

Criteria	
1	Family with ≤ 2 or more cases of gastric cancer with at least 1 diffuse gastric cancer (DGC)
2	Family with multiple lobular breast cancer with or without DGC in first degree relatives
3	Personal diagnosis of DGC <35 years of age from a low incidence population
4	Personal diagnosis of both DGC and lobular breast cancer
Other Possible Criteria	
1	Family with ≤3 cases of gastric cancer diagnosed at any age in which ≤1 is a DGC
2	Family with one or more cases of both DGC and signet ring adenocarcinomaof the colon

Adapted from Suriano et al. [40]

Table 4

Biomarkers for pancreatic neoplasia

Tumor	Biomarker	Category
Adenocarcinoma	CA19-9	Therapeutic Response; Prognosis
Pancreatic cystic neoplasms	Cyst fluid CEA	Diagnosis
	PathFinder TG	Diagnosis

Table 5

Biomarkers for colonic neoplasia

Disease	Biomarker	Category
Sporadic adenocarcinoma	Stool DNA	Diagnosis
	KRAS gene	Therapeutic Response
	MLH1 Methylation	Therapeutic Response
Familial adenomatouspolyposis	*APC* gene	Diagnosis
MUTYH-associated adenomatous polyposis	*MUTYH* gene	Diagnosis
Hereditary non-polyposis colorectal cancer	MSI	Diagnosis
	MLH1, MSH2, MSH6, PMS2 genes	Diagnosis
	MLH1Methylation	Therapeutic response