

2 RECENT ADVANCES IN HISTOPATHOLOGY OF GASTROINTESTINAL CANCERS: PROGNOSTIC AND THERAPEUTIC ASSESSMENT OF COLORECTAL CANCERS

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CHAPTER OVERVIEW

The genetics and molecular biology, precursor lesions and predisposing conditions, and hereditary syndromes of gastrointestinal cancers, especially colorectal cancers, are well characterized. Fifteen to twenty percent

of sporadic colorectal carcinomas have microsatellite instability (MSI; replication-error phenotype), characterized by defective DNA repair resulting in alterations of short tandem repeat sequences, including mononucleotide, dinucleotide, and tetranucleotide repeats. This is due to alteration of mismatch repair enzymes. Patients with colorectal cancers with MSI have a better prognosis than do those without. In contrast, about 50% to 60% of colorectal cancers have loss of the long arm (q) of chromosome 18, the chromosomal location of the *deleted in colorectal cancer*, *SMAD4*, and *SMAD2* genes. Chromosome 18q loss has been associated with poor outcome in patients with colorectal cancer. Growth factors and growth factor receptors play a major role in the development and progression of cancer. Gastrointestinal cancers express epidermal growth factor receptor (EGFR) and related receptors that activate intrinsic tyrosine kinase activity and result in signals of cell proliferation. This activity can be modulated by a variety of therapeutic options, including monoclonal antibody against EGFR or related receptors and selective inhibition of tyrosine kinase activity. Immunohistochemical analysis for EGFR can select patients who have EGFR-overexpressing gastrointestinal cancer and thus are potential candidates for anti-EGFR therapy.

INTRODUCTION

Among the most important recent advances in the field of gastrointestinal cancer are elucidation of the genetics of these cancers and characterization of the molecular pathways utilized by these neoplasms. This work has revolutionized many aspects of patient care, including prevention, screening, and treatment. The goal now is to identify new therapeutic targets that can be utilized for therapy and for increasing our understanding of prognosis. In cases of gastrointestinal cancer, histopathologic analysis typically provides diagnosis of predisposing conditions, information necessary for the surveillance of such conditions, and diagnosis of the type, grade, and stage of cancer. The evaluation of molecular predictors of prognosis and therapeutic response is a recent development in histopathology.

HISTOPATHOLOGIC FEATURES OF GASTROINTESTINAL CANCERS

Malignancies of the gastrointestinal tract can be classified histopathologically as epithelial tumors, endocrine or mesenchymal tumors, or lymphomas (Table 2-1; Hamilton and Aaltonen, 2001). Epithelial tumors can be subclassified as adenocarcinoma, squamous cell carcinoma, adenosquamous carcinoma, small cell carcinoma, carcinoid tumor, or other.

Table 2-1. Histopathologic Classification of Primary Malignant Neoplasms of the Esophagus, Stomach, and Colon and Rectum

Histopathologic Classification	Histopathologic Subtype by Anatomic Location			
	Esophagus	Stomach	Small Intestine	Colon and Rectum
Epithelial	Squamous cell carcinoma	Adenocarcinoma	Adenocarcinoma	Adenocarcinoma
	Verrucous (squamous) carcinoma	Intestinal type	Mucinous adenocarcinoma	Mucinous adenocarcinoma
	Basaloid squamous carcinoma	Diffuse type	Signet-ring-cell adenocarcinoma	Signet-ring-cell adenocarcinoma
	Spindle cell (squamous) carcinoma	Papillary adenocarcinoma	Adenosquamous carcinoma	Adenosquamous carcinoma
	Adenocarcinoma	Tubular adenocarcinoma	Squamous cell carcinoma	Squamous cell carcinoma
	Adenosquamous carcinoma	Mucinous	Small cell carcinoma	Small cell carcinoma
	Mucoepidermoid carcinoma	adenocarcinoma	Medullary carcinoma	Medullary carcinoma
	Adenoid cystic carcinoma	Signet-ring-cell adenocarcinoma	Undifferentiated carcinoma	Undifferentiated carcinoma
	Small cell carcinoma	Adenosquamous carcinoma	Other	Other
	Undifferentiated carcinoma	Squamous cell carcinoma		
	Other	Small cell carcinoma		
	Carcinoid tumor (well-differentiated endocrine neoplasm)	Undifferentiated carcinoma		
		Other		
		Carcinoid tumor (well-differentiated endocrine neoplasm)		
		Mixed carcinoid-adenocarcinoma		
Endocrine		Carcinoid tumor (well-differentiated endocrine neoplasm)		
		Mixed carcinoid-adenocarcinoma		

Carcinoid tumor (well-differentiated endocrine neoplasm)

Mixed carcinoid-adenocarcinoma

Nonepithelial Mesenchymal	Gastrointestinal stromal tumor Leiomyosarcoma Rhabdomyosarcoma Kaposi's sarcoma Malignant melanoma Other	Gastrointestinal stromal tumor Leiomyosarcoma Angiosarcoma Kaposi's sarcoma Other	Gastrointestinal stromal tumor Leiomyosarcoma Angiosarcoma Kaposi's sarcoma Other
Malignant Lymphomas		Immunoproliferative small intestinal disease (includes α -heavy-chain disease) Marginal zone B-cell lymphoma of MALT type Mantle cell lymphoma Diffuse large B-cell lymphoma Burkitt's lymphoma Burkitt's-like/atypical Burkitt's lymphoma Enteropathy-associated T-cell lymphoma Other	Marginal zone B-cell lymphoma of MALT type Mantle cell lymphoma Diffuse large B-cell lymphoma Burkitt's lymphoma Burkitt's-like/atypical Burkitt's lymphoma Other

Adenocarcinoma can be further subclassified as adenocarcinoma, not otherwise specified, or intestinal, signet-ring-cell, or mucinous (colloid) adenocarcinoma.

Tumor stage at the time of diagnosis is the most important factor in determining prognosis. Current TNM staging systems for gastrointestinal cancer are shown in chapter 1 (Tables 1–1 through 1–6) and chapter 15 (Tables 15–1 and 15–2). The 5-year survival rates for patients with gastrointestinal cancer differ by anatomic site and histologic subtype of cancer.

METASTATIC CARCINOMA OF UNKNOWN PRIMARY ORIGIN

In most patients, the site of origin of a metastatic carcinoma of unknown primary origin cannot be reliably determined by light microscopy (Hammar, 1998). Almost 60% of metastatic carcinomas of unknown primary origin are adenocarcinomas. Some metastatic adenocarcinomas (e.g., colonic adenocarcinomas) have distinctive histologic features that allow for determination of their site of origin. For most other metastatic adenocarcinomas of unknown primary origin, immunohistochemical analysis can help to identify the primary site. Immunophenotyping for cytokeratin 7, cytokeratin 20 (Chu et al, 2000), and other antigens used in conjunction with histologic analysis is effective in narrowing the potential primary site of origin of adenocarcinomas (Table 2–2), although these and other antigens are not absolutely site specific and cannot be reliably used to determine the site of origin. Other antigens that help determine the site of origin are thyroglobulin for thyroid, prostate-specific antigen and prostatic alkaline phosphatase for prostate, estrogen receptor for breast, and thyroid transcription factor 1 for lung and thyroid. Thyroglobulin, prostate-specific antigen, and prostatic alkaline phosphatase are site specific. Ultrastructural details of neoplastic cells can be studied by electron microscopy and may help determine the tumor type and site of origin of poorly differentiated cancers.

GENETIC ALTERATIONS OF COLORECTAL CANCER

The molecular genetic alterations in colorectal carcinoma are among the best understood in human cancer and involve abnormalities in multiple dominant-acting oncogenes and tumor-suppressor genes (Kinzler and Vogelstein, 1996; Fearon and Dang, 1999). Various pathways of colorectal carcinogenesis are evident in sporadic, familial, and inflammatory bowel disease-associated neoplasms. The somatic alterations in sporadic colo-

Table 2-2. Immunophenotype of Various Adenocarcinomas

<i>Immunophenotype</i>		<i>Tumors by Site and Type</i>
<i>Cytokeratin 7</i>	<i>Cytokeratin 20</i>	
Positive	Positive	93% of ovarian mucinous carcinomas 62% of pancreatic adenocarcinomas 43% of cholangiocarcinomas 25% of bladder transitional cell carcinomas 13% of gastric adenocarcinomas
Positive	Negative	100% of salivary gland carcinomas 98% of thyroid carcinomas 96% of breast carcinomas 96% of ovarian endometrioid, serous, and clear cell carcinomas 80% of endometrial endometrioid carcinomas 72% of lung carcinomas 65% of malignant mesotheliomas
Negative	Positive	95% of colorectal carcinomas 78% of Merkel cell tumors of skin 37% of gastric carcinomas
Negative	Negative	100% of prostatic carcinomas 89% of renal cell carcinomas 81% of hepatocellular carcinomas 79% of pulmonary and gastrointestinal carcinoid tumors

rectal carcinoma include truncating mutations or deletions of the *adenomatous polyposis coli (APC)* gene on chromosome 5q and mutations of the *β-catenin* gene. Point mutations of the *K-ras* proto-oncogene, loss of the *deleted in colorectal cancer* gene and nearby *SMAD2* and *SMAD4* genes on chromosome 18q, and mutations and deletions of the *p53* gene on chromosome 17p are also common. Familial adenomatous polyposis is an autosomal-dominant inherited syndrome characterized morphologically by more than 100 colorectal adenomas and is due to a germline mutation in the *APC* gene. The tumors have somatic alterations similar to those of sporadic cancers.

In a second pathway to colorectal neoplasia, microsatellite instability (MSI; also termed DNA replication errors and ubiquitous somatic mutations) is caused by the alteration of a nucleotide mismatch repair gene, including *hMSH2*, *hMLH1*, *PMS1*, *PMS2*, or *GTBP*. MSI is characterized by additions and deletions of nucleotides in numerous repeated nucleotide sequences (microsatellites). Germline mutation of a mismatch repair gene causes hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal-dominant syndrome characterized by early-onset, right-

sided, familial colorectal cancer. Affected individuals have a tendency to develop synchronous and metachronous lesions and have an increased incidence of endometrial, ovarian, gastric, small bowel, and renal pelvic cancer. Silencing of the *hMLH1* gene by methylation is common in sporadic MSI-positive cancers. Alterations of mononucleotide tracts that are present in *transforming growth factor β type II receptor* and *BAX* are commonly found in MSI-positive carcinomas. MSI-positive colorectal carcinomas are more commonly right-sided with diploid total DNA content and are associated with slightly better patient survival than are MSI-negative cancers. Sporadic gastric and endometrial cancers also commonly are MSI positive.

Recently, a distinct pathway of colorectal carcinogenesis, termed CpG island methylator phenotype (CIMP), was described (Toyota et al, 2000). CIMP-positive colorectal cancers are characterized by a high degree of concordant CpG island methylation of multiple genes and loci in the tumor that are not methylated in normal colorectal mucosa (Baylin et al, 1998). CpG islands are 0.5- to 2.0-kilobase regions rich in the cytosine-guanine dinucleotides and are present in the 5' region of about half of all human genes. The methylation of cytosines within CpG islands is associated with loss of gene expression by repression of transcription and is observed not only in physiologic conditions such as X chromosome inactivation and aging but also in neoplasia. Examples of this process in colorectal cancer include inactivation of the *p16* cell-cycle regulator, the *THBS1* angiogenesis inhibitor, the *TIMP3* metastasis suppressor, the *O⁶-methylguanine DNA methyltransferase* DNA repair gene, and the *hMLH1* nucleotide mismatch repair gene. Most sporadic MSI-high colorectal cancers (tumors in which 2 or more defined markers show instability) are due to methylation of the *hMLH1* mismatch repair gene.

HISTOPATHOLOGIC FEATURES OF COLORECTAL CANCERS WITH MSI

MSI-high colorectal cancers have a distinct clinicopathologic phenotype (Kim et al, 1994; Jass et al, 1998; Alexander et al, 2001). MSI-high colorectal cancers are more frequent in younger patients. Most are right-sided (proximal to the splenic flexure), bulky (large) tumors, with an exophytic growth pattern; are poorly differentiated, with signet-ring-cell, mucinous, medullary, or variegated (mixed) histologic subtypes; have an intense lymphocytic response with Crohn's-like lymphoid reaction (lymphoid follicles with germinal centers at the tumor edge) and peritumoral and intratumoral lymphocytosis; and show an expanding (pushing) invasive pattern at the margins. However, one third of colorectal carcinomas with MSI do not have these histologic characteristics.

PROGNOSTIC SIGNIFICANCE OF CHROMOSOME 18Q LOSS AND MSI

Chromosome 18q loss (Jen et al, 1994) and MSI (Gryfe et al, 2000) are prognostic factors for sporadic colorectal cancer. In a study of 319 patients with stage III colon cancer, chromosome 18q loss was associated with a worse prognosis after chemotherapy (Watanabe et al, 2001). Patients with tumors that retained chromosome 18q had a 5-year overall survival rate of 69%, compared with 50% for patients with loss of heterozygosity at chromosome 18q. Similarly, patients with sporadic MSI-positive colorectal cancers had a better prognosis. In a population-based study of 587 patients 50 years of age or younger, MSI-high cancers were associated with better survival and decreased likelihood of metastasis to regional lymph nodes or distant organs (Gryfe et al, 2000). The 5-year survival rate was 76% for patients with MSI-high colorectal cancers but 64% for those with microsatellite-stable cancers. The MSI-high phenotype is less frequent in metastatic colorectal cancers. MSI is also a prognostic factor for gastric cancers: MSI-high gastric cancers are associated with a better survival than are microsatellite-stable gastric cancers.

CHROMOSOME 18Q LOSS AND MSI ASSAY IN SURGICAL PATHOLOGY PRACTICE

Chromosome 18q loss and MSI can be assessed by using archival blocks of surgically resected tumors that have been fixed in formalin and embedded in paraffin. At M. D. Anderson Cancer Center, these assays are performed by the Diagnostic Molecular Laboratory. The tumor and normal tissue are microdissected, and DNA is extracted by proteinase K digestion. Genomic DNA is used to amplify sequences by polymerase chain reaction (PCR) using 5 markers (oligonucleotides that can amplify microsatellite repeats) present on chromosome 18q for chromosome 18q loss analysis, and 5 markers recommended by the National Cancer Institute workshop for MSI assay (Boland et al, 1998). The markers for MSI assay include 2 mononucleotide markers, BAT-25 and BAT-26, and 3 dinucleotide markers, D2S123, D5S346, and D17S250. The PCR products are electrophoresed on an automated sequencer. Chromosome 18q loss is assessed by allelic loss of a polymorphic (with 2 alleles) marker indicating loss of 1 copy of chromosome 18q. The presence of an additional band in the PCR product from tumor DNA, not observed in DNA from normal tissue from the same patient, is scored as an allelic shift (instability) at that locus. In accordance with the National Cancer Institute consensus on MSI, any pair of samples of normal DNA and tumor DNA that displays instability at 2 or more of 5 loci is scored as MSI high; any pair that displays instability

at 1 locus is scored as MSI low; and any pair that displays no instability at 5 loci is scored as microsatellite stable.

IMMUNOHISTOCHEMISTRY FOR MISMATCH REPAIR GENES

Mismatch repair genes are tumor-suppressor genes, and loss or inactivation of both alleles is required in tumors. Detection of loss of expression of *hMLH1*, *hMSH2*, or *hMSH6* by immunohistochemical analysis (Figure 2–1) can help to identify MSI-high cancers. Most sporadic MSI-high cancers have loss of expression of *hMLH1* due to hypermethylation of the gene (Thibodeau et al, 1998). The detection of the loss of a mismatch repair gene, in conjunction with the age and family history of the patient, can help determine appropriate management. Patients who are young at the onset of colorectal cancer or who have a family history of colorectal cancer may be tested for a germline mutation and undergo genetic counseling. On the other hand, colorectal cancer in patients who are older at the onset of disease, have no family history of colorectal cancer, and demonstrate loss of *hMLH1* by immunohistochemical analysis is probably due to hypermethylation of the *hMLH1* promoter site, and testing for a germline mutation is not indicated.

INDICATIONS FOR MSI ASSAY

The indications for an MSI assay are listed in chapter 10 (Table 10–1). The primary reason to perform an MSI assay is to rule out HNPCC. HNPCC kindreds may develop multiple cancers of the colorectum or other sites, and the operation of choice in affected individuals is pan-colectomy. Surveillance for cancers of other sites should be considered. Family members of patients with HNPCC are at risk, and genetic counseling for these individuals is recommended. The second reason to perform an MSI assay is that the MSI phenotype in colorectal cancer has prognostic significance.

The International Collaborative Group on HNPCC has established minimal criteria for identifying patients with HNPCC. These criteria are known as the Amsterdam criteria and are as follows: (1) at least 3 relatives with histologically verified colorectal cancer, 1 of them a first-degree relative of the other 2; (2) at least 2 successive generations affected; and (3) in 1 of the individuals, colorectal cancer should have been diagnosed before the age of 50 years.

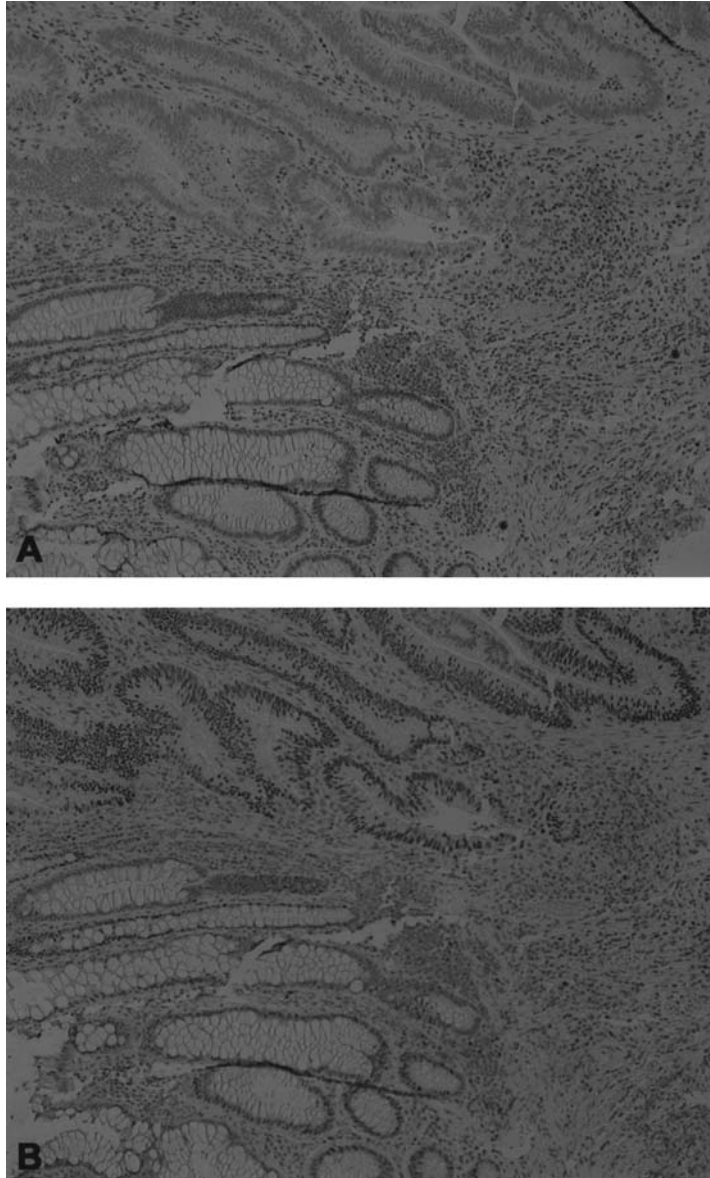


Figure 2-1. Immunohistochemical analysis for hMLH1 (panel A) and hMSH2 (panel B). There is nuclear staining of the epithelium of normal mucosa (lower half of both panels) and lymphocytes and stromal cells. Colon carcinoma (upper half of both panels) has loss of expression of hMLH1, but hMSH2 is expressed. Loss of a mismatch repair gene is invariably associated with microsatellite instability in tumors.

THERAPEUTIC IMPORTANCE OF OVEREXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR AND RELATED RECEPTORS

The epidermal growth factor and related polypeptides, including transforming growth factor α , amphiregulin, heparin-binding epidermal growth factor-like growth factor, betacellulin, and epiregulin, are synthesized as propeptides containing a cytoplasmic domain, a transmembrane domain, and an extracellular domain. Metalloproteases proteolytically release mature peptides from the extracellular domain. These peptides bind epidermal growth factor receptor (EGFR) and related receptors. There are 4 members of the human EGFR family: human epidermal growth factor receptor 1 (HER1, also known as ErbB1), ErbB2 (HER2 or neu), ErbB3 (HER3), and ErbB4 (HER4). These proteins are membrane-associated receptor tyrosine kinases with an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain that has intrinsic tyrosine kinase activity. The tyrosine kinase activity is activated by ligand-induced receptor homodimerization and heterodimerization. No ligand has been shown to bind directly to ErbB2. Instead, ErbB2 functions as a coreceptor, forms heterodimers with other members of the EGFR family, and increases the affinity of ligands for the receptor complex. Activated homodimeric or heterodimeric receptors recruit proteins with *Src* homology 2 domains. Ras and phospholipase C- γ signaling pathways are activated by the activation of the EGFR. The end result of signaling by these pathways is stimulation of cellular proliferation, enhancement of cell survival, modulation of cell migration, and adhesion.

ErbB1, ErbB2, and ErbB3 are overexpressed in gastrointestinal malignancies, including colorectal, esophageal, gastric, pancreatic, and hepatocellular cancers. Overexpression or amplification of these receptors in gastrointestinal cancers correlates with a poor prognosis and aggressive disease.

Blockage of epidermal growth factor signaling may reduce the growth of malignant cells. A variety of agents—including transforming growth factor- α and EGFR-neutralizing antibodies, epidermal growth factor-related peptide antisense constructs, ADAM metalloprotease inhibitors, and epidermal growth factor tyrosine kinase inhibitors—have been utilized to block the epidermal growth factor pathway (Barnard, 2001). Human clinical trials using anti-ErbB2 monoclonal antibody (trastuzumab, Herceptin; Genentech, Inc., South San Francisco, CA), anti-human EGFR antibody (ICM-C225), and EGFR tyrosine kinase inhibitor (OSI-774) have been reported.

IMMUNOHISTOCHEMISTRY FOR EGFR AND RELATED RECEPTORS

Overexpression of EGFR and related receptors can be assessed by immunohistochemical analysis using formalin-fixed, paraffin-embedded tissue (Figure 2–2). At M. D. Anderson, EGFR expression is assayed using mouse monoclonal antibody 31G7 (Zymed Laboratories, San Francisco, CA), and ErbB2 expression is assayed using AD8 monoclonal antibody (NeoMarkers, Lab Vision Corporation, Fremont, CA). The intensity of EGFR reactivity is scored as follows: 0, no reactivity or cytoplasmic staining of neoplastic cells; 1+, weak or faint, discontinuous, membranous staining of neoplastic cells; 2+, intermediate, incomplete, membranous staining of neoplastic cells; and 3+, intense, continuous membranous staining (Goldstein and Armin, 2001). The percentage of immunoreactive cells also is reported. In a study of EGFR immunohistochemical reactivity in colon cancer, 31.4% of neoplastic cells had 3+ reactivity in more than 10% to 50% of the neoplastic cells, and 3.9% of neoplastic cells had 3+ reactivity in more than 50% of the neoplastic cells (Goldstein and Armin, 2001). Overexpression of ErbB2 is due to amplification of the *ErbB2* gene. This can be corroborated by fluorescence in situ hybridization analysis.

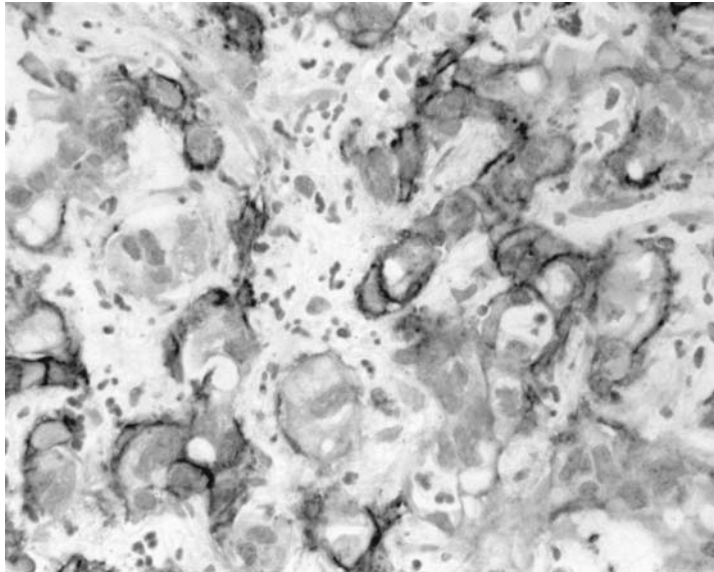


Figure 2–2. Immunohistochemical analysis for epidermal growth factor receptor. Metastatic colon cancer has an intense, continuous, membranous staining (3+) for epidermal growth factor receptor.

KEY PRACTICE POINTS

- Chromosome 18q loss and MSI are prognostic factors for patients with colorectal cancer.
- MSI assay and immunohistochemical analysis for mismatch repair genes, in conjunction with the age and family history of the patient, can help to differentiate sporadic from HNPCC-associated colorectal carcinomas.
- Overexpression of EGFR can be assessed by immunohistochemical analysis and can help select patients who will benefit from therapy against EGFR.

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