Newer Pathologic Assessment Techniques for Colorectal Carcinoma

Roderick R. Turner,1 Carolyn Li,2 and Carolyn C. Compton3

Abstract

The pathogenesis of colorectal carcinoma is characterized by progressive genetic abnormalities, which lead to proteomic and cellular changes that determine the cancer malignant phenotype. Phenotypic characteristics seen on histopathologic examination (e.g., tumor stage, histologic grade, and vasoinvasiveness) are essential to planning patient management and should continue to be the major focus of pathologists’ efforts. Nonetheless, additional markers that improve the prognostic and predictive power of the pathologic analysis of the primary tumor have been the focus of intense research in recent years. Improved prognostic power may derive from advancements in histopathologic evaluation, more sensitive lymph node staging techniques, and specific molecular analysis methods, such as genetic tests or immunophenotypic profiles. Histopathologic improvements are needed to better standardize histologic gradedetermination and recognize tumor budding at the invasive front as a marker of aggressive biological behavior and an adverse parameter. Ultrastaging of mesenteric lymph nodes remains a controversial area. Genotypic studies are well developed in the areas of microsatellite instability and chromosome 18q deletion/loss of heterozygosity. Immunophenotypic studies are available in a range of areas including tumor suppressor gene/oncogene expression, proliferation/apoptosis, angiogenesis, and cell adhesion and signaling. Gene expression profiles identified by microarray techniques may help to subtype the large category of microsatellite-stable colorectal carcinoma and define immunophenotypic panels to subclassify tumors into prognostic and therapeutic groups. This brief review discusses the most promising of these approaches and evidence supporting their potential clinical utility.

Pathologists are being asked to develop prognostic marker assays to identify high-risk patients with colorectal carcinoma who may benefit from adjuvant therapies. In recent years, this area of research has been active and exciting, and substantial progress has been made in our understanding of colorectal carcinoma development and metastasis (1, 2).

The overall pathogenesis of colorectal carcinoma is characterized by progressive genetic abnormalities (3) that are variable in type and temporal development in any given case. Specific DNA mutations or epigenomic abnormalities in a cancer lead to cellular protein abnormalities that alter cell cycle regulatory mechanisms (4). It is expected that knowledge of the specific genetic, proteomic, and other cell biological abnormalities of a tumor can be used to improve both prediction of biological behavior and the prognostic power of standard tumor-node-metastasis stage and histologic grade. Such knowledge would facilitate identification of patients at high risk of adverse outcome (recurrence or death) who might merit more aggressive treatment and prediction of response to specific therapies.

Approaches that are not yet standard practice but may improve prognosis in colorectal carcinoma include analysis of additional histopathologic factors, advanced lymph node staging with immunohistochemistry or reverse transcription-PCR (RT-PCR), and primary carcinoma genotypic and phenotypic studies. This brief review summarizes the current status of these newer approaches.

Histopathologic Factors: Primary Tumor

Tumor grade is recognized as an important prognostic parameter in colorectal carcinoma, but at present, there is no consensus on a grading system. The College of American Pathologists has recommended a two-tier system (5), whereas the WHO has recommended a four-tier system (6). Both systems are based solely on percentage of gland formation (Table 1). Others have suggested that grade assessment by the worst pattern (regardless of relative amount) may correlate more significantly with clinical outcome (7). Nuclear grade in colorectal carcinoma is generally not reported, although it has been suggested that low-grade adenocarcinomas should not have a significant component with high nuclear grade (8).

Tumor budding, also known as dedifferentiation, is a recently recognized feature that represents a high-grade, undifferentiated component of a tumor at the leading invasive edge. Described by Morodomi et al. (9) as small clusters or single infiltrating carcinoma cells (<5) at the invasive edge,
tumor budding may predict high risk of recurrence after curative surgery and indirectly provides support for grading by the worst pattern (Fig. 1). This finding correlates with loss of adhesion molecule expression (10) and increased metalloprotease expression (11), corresponding to a more aggressive tumor phenotype on a molecular level. Because these cell clusters may be quite small and do not form glands or produce mucin, identification on histopathologic examination may be difficult. Cytokeratin immunohistochemical stains may be helpful in their identification, especially if accompanied by an inflammatory reaction that obscures their presence on H&E staining (ref. 12; Fig. 2). The survival rate in stage II patients with tumor budding may not be significantly different than that in all stage III patients (13). Nonetheless, the amount of tumor budding also seems to be important. Prominent tumor budding correlates strongly with adverse outcome, whereas a minimal or focal finding may not (14). This feature needs to be better defined because the frequency of tumor budding varies widely in the literature (i.e., from 20% to 89%; refs. 14, 15). Tumor budding is distinct from tumor border configuration. The latter is classified as either pushing (smooth) or infiltrating (jagged), which is a low-magnification tumor architectural feature shown in several studies to be a stage-independent adverse prognostic factor (16–19). Tumor budding may occur in tumors with either a pushing or an infiltrating border, but is more often associated with the latter.

### Lymph Node Special Techniques

**Background.** The current minimum standard for pathologic examination of regional lymph nodes in a surgical resection specimen (20) is 12 lymph nodes; however, examination of a greater number of nodes provides better prognostic information in both node-positive and node-negative diseases (21). Survival rates have been shown to decrease with increasing numbers of positive lymph nodes. Nodal metastasis is commonly small, and evidence suggests that patients with micrometastasis in multiple nodes by H&E examination may have an outcome similar to patients with multiple macrometastasis (22). Conversely, high numbers of negative lymph nodes have been correlated with better survival. These findings have been presumed to be the result of more sensitive and accurate lymph node staging, with fewer false-negative results; however, an explanation for the association with improved survival is not yet clear (23). Other improvements in nodal staging have been directed toward the detection of occult tumor cells with immunohistochemistry techniques or molecular evidence of tumor cells by RT-PCR.

**Immunohistochemistry.** Cytokeratin immunohistochemistry provides a sensitive (but not entirely specific) tool for detecting occult tumor cells in mesenteric lymph nodes, with positive conversion rate in ~25% to 30% of patients with colorectal carcinoma. Published studies presenting these data preceded the era of isolated tumor cells, which were described as micrometastasis; however, most of these findings presumably represent today’s isolated tumor cell category (up to 0.20 mm). Some studies have found a clinically significant association between nodal tumor cells and shorter survival (24–27), whereas others have not (refs. 28–32; Table 2). Primary tumor characteristics, such as histologic grade (32) or molecular markers (33), have not yet been shown to correlate with nodal tumor cells. Recent evidence suggests that tumor budding is associated with both lymphatic invasion and nodal tumor cells (12).

Technical issues related to immunohistochemical detection of isolated tumor cells have included number of histologic levels needed, choice of antibodies, and criteria for positivity. Limited step section examination at two or three levels seems to be sufficient (30, 34, 35) because tumor cell clusters or single cells are commonly found at multiple levels of lymph nodes. Most studies have used a pan-cytokeratin, either AE-1/AE-3 or Cam 5.2, whereas others have used cytokeratin 20 or carcinoembryonic antigen antibodies as more specific, albeit slightly less sensitive, markers of colonic epithelium (26, 28). An attractive feature of immunohistochemistry analysis is that it permits direct microscopic visualization of immunoreactive cells, which may help to exclude cellular debris in macrophages or other contaminants (Fig. 3). Criteria for cytokeratin positivity that exclude rare single cells without malignant cytologic features have been proposed (35).
Molecular analysis. RT-PCR techniques have been used to detect submicroscopic molecular evidence of isolated tumor cells in lymph nodes, with positive results in ~40% to 50% of patients. Early studies, predominantly using carcinoembrionic antigen and cytokeratin-20 probes, have shown good correlation with worse survival (36–38). Until recently, these techniques have required fresh or frozen tissue—in some cases, consuming half of the lymph nodes examined, which has generated concern about the comparison of RT-PCR results with standard histopathologic staging. Paraffin-embedded tissue techniques, now available, make this assay more practical. Other concerns about RT-PCR include high sensitivity and variable amplification, which may cause false-positive results, and the absence of microscopic identification of the positive signal.

A meta-analysis comparing immunohistochemistry and RT-PCR detection of isolated tumor cells favored RT-PCR (39), but was based on few studies, relatively small patient populations, and short (3-year) survival calculations. Data generated from studies combining larger numbers of submitted lymph nodes and better immunohistochemistry techniques would be a more reliable indicator of the significance of isolated tumor cell detection.

In summary, enhanced detection of isolated tumor cells with either immunohistochemistry or RT-PCR needs further evaluation in larger prospective trials to determine the potential role of these techniques in the prognosis and selection of therapies.

Specific lymph node identification. Lymphatic mapping and sentinel lymph node identification may help the surgeon identify lymphatic drainage routes, including those lymph nodes in the resection, and permit the pathologist to apply ultrastaging techniques to first-draining lymph nodes. Although some studies have suggested that this procedure is useful in micrometastasis detection (40–44), others have highlighted the lack of established techniques and high false-negative rates (45). One important observation with the sentinel lymph node procedure in colorectal carcinoma is that few patients (usually <20%) are sentinel lymph node-only positive (46). The high rate of nonsentinel lymph node metastasis, with or without sentinel lymph node metastasis, may be attributed to the rich anastomotic relationship of lymphatics in colonic mesentery.

Table 2. Selected nodal immunohistochemistry studies

<table>
<thead>
<tr>
<th>Published study (reference citation)</th>
<th>No. patients</th>
<th>No. lymph nodes</th>
<th>Percentage positive</th>
<th>Duration of follow-up (y)</th>
<th>Prognostic value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutait et al. (28)</td>
<td>46</td>
<td>13</td>
<td>26</td>
<td>5</td>
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<tr>
<td>Jeffers et al. (29)</td>
<td>77</td>
<td>7</td>
<td>25</td>
<td>10</td>
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<tr>
<td>Greenson et al. (24)</td>
<td>50</td>
<td>11</td>
<td>28</td>
<td>5</td>
<td>Yes</td>
</tr>
<tr>
<td>Adell et al. (30)</td>
<td>100</td>
<td>4</td>
<td>39</td>
<td>5</td>
<td>No</td>
</tr>
<tr>
<td>Clarke et al. (25)</td>
<td>133</td>
<td>7</td>
<td>25</td>
<td>5</td>
<td>Yes</td>
</tr>
<tr>
<td>Yasuda et al. (27)</td>
<td>42</td>
<td>18</td>
<td>76</td>
<td>Not reported</td>
<td>Yes</td>
</tr>
<tr>
<td>Choi et al. (31)</td>
<td>93</td>
<td>15</td>
<td>31</td>
<td>5</td>
<td>No</td>
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<tr>
<td>Fisher et al. (32)</td>
<td>399</td>
<td>Not reported</td>
<td>18</td>
<td>10</td>
<td>No</td>
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<tr>
<td>Rosenberg et al. (26)</td>
<td>85</td>
<td>Not reported</td>
<td>27</td>
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For micrometastasis/isolated tumor cell detection, greater accuracy is achieved by applying the special techniques to all lymph node blocks. Overall, the data are still insufficient to support routine use of specialized techniques for lymph node mapping or sentinel lymph node analysis (47).

**Primary Tumor Markers**

**Background.** Molecular testing of the primary colorectal carcinoma tumor holds great promise and has been the subject of intensive efforts in recent years for determining how to better predict risk of disease progression. Many methods and factors are under investigation, the more common of which are shown in Table 3. An important prognostic distinction is made between tumors that show abnormalities in mismatch repair genes and the larger category of tumors in the chromosomal instability pathway.

**Table 3. Potential molecular markers for primary colorectal cancer**

<table>
<thead>
<tr>
<th>Marker Type</th>
<th>Example</th>
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<tr>
<td>MSI (RT-PCR, immunohistochemistry)</td>
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<tr>
<td>Allelic imbalances/loss of heterozygosity (18q-)</td>
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<tr>
<td>Chromosomal instability (DNA ploidy)</td>
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<tr>
<td>Methylation (gene silencing, genome-wide or specific)</td>
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<tr>
<td>Oncogene expression (ras, myc)</td>
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<tr>
<td>Tumor suppressor gene loss (bcl-2, p21, p27, p53)</td>
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<tr>
<td>Proliferation/apoptosis (bcl-2, bax, Ki-67)</td>
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<tr>
<td>Angiogenesis (vascular endothelial growth factor, D2-40, CD31)</td>
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<td>Inflammation (cyclooxygenase 2)</td>
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<tr>
<td>Cell adhesion (e-cadherin, b-catenin, CD44)</td>
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<tr>
<td>Specific predictive markers (e.g., epidermal growth factor receptor, thymidylate synthase, vascular endothelial growth factor, etc.)</td>
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**Microsatellite instability pathway.** Testing for mismatch repair status is well developed for clinical use with microsatellite instability (MSI) assays. MSI testing serves as an important prognostic and therapeutic marker as well as a screening tool for hereditary nonpolyposis colon cancer. MSI testing by RT-PCR, using the National Cancer Institute–recommended five-marker primer set, is regarded as the gold standard. However, immunohistochemistry offers an excellent alternative or complementary technique (48), and algorithms for use of both techniques have been published. Immunohistochemistry for MLH1 and MSH2 mutations provides a sensitivity of ~90% to 95% and a specificity close to 100%. Additionally, the technique identifies a protein epitope but fails to detect some mutations that encode a nonfunctional protein.

A constellation of histologic findings associated with MSI-H, as outlined in the revised Bethesda guidelines (49), could be used for selective testing of MSI-high (MSI-H) and its associated better prognosis (50). Histologic features related to MSI-H include mucinous, signet-ring cell, or medullary type; poor differentiation; tumor-infiltrating lymphocytes or Crohn’s-like reaction; and pushing tumor border (51). With the exception of tumor-infiltrating lymphocytes (52), however, these histologic features are nonspecific and, even when combined with younger age (revised Bethesda guidelines), have been associated with MSI-H in only 23% of cases (53). Therefore, H&E morphologic evaluation offers a low positive predictive value for mismatch repair status. Specific testing for molecular evidence of MSI-H– or immunohistochemistry-detected protein abnormalities is necessary to reliably determine mismatch repair status.

**Chromosomal instability pathway.** The chromosomal instability of colorectal carcinoma pathogenesis, ~85% of colorectal carcinomas, represents the main target for development of specific prognostic and predictive marker assays. Multiple
sequential molecular events causing oncogene mutations and loss of tumor suppressor genes lead to cell cycle dysregulation and inhibition of apoptosis. Deletion or loss of heterozygosity of a portion of chromosome 18 (18q-) is found in ~70% of colorectal carcinomas. This feature is linked to poor prognosis, which is attributed to loss of tumor suppressor genes in that region (2). Abnormalities of chromosome 17p are also common and result in mutation or deletion of p53. Overexpression of the mutant p53 protein is found by immunohistochemical staining in ~40% to 60% of colorectal carcinoma cases (54).

**Prognostic/predictive markers.** The 2006 update of the American Society of Clinical Oncology guidelines assessed a large number of prognostic/predictive markers and determined that none is currently recommended for clinical use (55). The American Society of Clinical Oncology recommendations are based on a comprehensive review of the literature but limited by individual assessment of each marker.

A better approach might be to use multiple markers that can better determine a tumor profile; early efforts suggest that this can be an effective tactic (56–58). DNA microarrays are also likely to be helpful in finding patterns or profiles of colorectal carcinoma with significant prognostic and predictive information. To date, gene expression profiling has pointed to different areas of importance. Fredericksen et al. (59) have suggested mitochondrial, stromal remodeling, and cell adhesion gene differences. Kwon et al. (60) have reported differential expression in signal transduction, cell structure/motility, and cell cycle. Bianchini et al. (61) have observed differences in apoptotic inhibitors, up-regulation of HLA-E, and down-regulation of β₂-microglobulin in the future, important findings from DNA profiles may lead to small panels of immunohistochemistry markers that can reliably categorize colorectal carcinoma. Until the basic biological aspects of colorectal carcinoma with low-risk and high-risk profiles are better understood, rational decisions on therapies and efficacy of therapeutic trials will not reach their full potential.

**Summary**

- Tumor budding selects high-risk stage II cases.
- Refinements in histologic grading may further enhance its value.
- Detection of occult nodal metastasis warrants further evaluation.
- MSI-H identifies a better prognosis subset.
- Immunophenotypic studies of colorectal carcinoma should focus on marker panels that identify favorable and unfavorable groups.
- DNA microarrays look promising.
- Predictive marker immunohistochemistry assays are improving.

### References


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