Abstract

The basic mechanisms driving genetic instability underlie a new molecular classification of colorectal cancer that is assuming diagnostic and prognostic importance. These mechanisms and the criteria for stratifying colorectal cancer as microsatellite stable (MSS), microsatellite instability-low (MSI-L) and microsatellite instability-high (MSI-H) are presented. This molecular classification is discussed in relation to morphogenesis, histopathology, behaviour and investigation of prognostic biomarkers in colorectal cancer. Clinical applications are considered, emphasising the role of the pathologist in identifying and working up cases of suspected hereditary non-polyposis colorectal cancer. The principal value of microsatellite instability testing is in relation to the diagnosis of hereditary non-polyposis colorectal cancer. Demonstration of loss of DNA mismatch repair genes, notably $hMLH1$ and $hMSH2$, by immunohistochemistry provides additional diagnostic information and may reduce the requirement for microsatellite instability testing. It is likely that testing for DNA mismatch repair will be adopted as a routine for colorectal cancer as more is learned of the distinctive pathobiology and behaviour of MSS, MSI-L and MSI-H cancers.

Key words

Colorectal · Cancer · Molecular genetics · Microsatellite instability (MSI) · Classification

Introduction

Despite the rapid proliferation of molecular biological data, the impact of these advances upon the work of the diagnostic histopathologist has been limited to a few specialist areas. It has been pointed out that the classification of epithelial neoplasms has been largely unaffected by genetic discoveries [1]. Although various molecular pathways are now known to exist in the case of colorectal cancer, early work in this area suggested that these pathways are not clearly distinguished from one another but show overlapping molecular characteristics [2]. More recent insights into the mechanisms underlying genetic instability indicate that several distinct molecular pathways exist, and that molecular classifications of colorectal cancer are likely to assume increasing clinical importance [3]. The evidence is reviewed in this contribution.

Genetic instability

It is now well known that the malignant phenotype of solid tumours depends on the acquisition of multiple genetic changes [4]. It has been suggested that the generation of the requisite mutations depends on the prior establishment of a state of genetic instability [5]. In colorectal cancer two types of genetic instability are recognised. These are manifested by the subtle sequence changes known as DNA microsatellite instability (MSI) due to an underlying defect in DNA mismatch repair and, secondly, by high rates of chromosome losses and gains associated with chromosomal instability [6].

At least five DNA mismatch repair genes are known to be associated with DNA MSI in human colorectal cancer. Of these, $hMLH1$, $hMSH2$ and $hMSH6$ are the most frequently implicated in hereditary non-polyposis colorectal cancer (HNPPC) [7]. In sporadic colorectal cancer showing DNA MSI, $hMLH1$ is the most frequently inactivated gene, and this is often achieved by hypermethylation of the promoter region [8]. Inactivation of both copies of a DNA mismatch repair gene manifests in the failure to repair mismatches that arise during the course of DNA synthesis. Mismatches are most likely to occur in repetitive sequences which are common in non-coding microsatellites. Some genes contain short repetitive sequences and are therefore at risk of mutation. Genes that may be targeted by this mechanism and are found to be mutated in MSI colorectal cancer include

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Testing for DNA microsatellite instability

It is now possible to separate colorectal cancers into those showing DNA microsatellite instability and those that are microsatellite stable (MSS). A recent National Cancer Institute workshop recommended the use of a reference panel of two mononucleotide markers (BAT25, BAT26) and three dinucleotide markers (D5S346, D2S123 and D17S250) [17]. The same workshop emphasised the distinction between low and high levels of MSI. When large numbers of microsatellite markers are tested, the distribution of MSI cancers is clearly bimodal [3, 18]. In the MSI-low (L) group less than 40% of markers show instability whereas over 40% show instability in the case of MSI-high (H) cancers. With respect to the recommended panel, MSI-H is diagnosed when at least two of the five loci show instability. The distinction between MSI-H and MSI-L is not merely quantitative. Both forms appear to be distinguishable are these two mechanisms in practice?

Determinants of MSI-L versus MSI-H

DNA mismatch repair requires the cooperation of one of several sets of DNA mismatch repair proteins. While the precise explanation for loss of DNA repair proficiency is still being elucidated, it is likely that loss of one functioning repair enzyme system leads to a less severe DNA repair defect whereas loss of two (or more) systems leads to a more severe repair defect. The DNA repair enzymes hMSH3 and hMSH6 contain repetitive tracts that are susceptible to replication errors. Mutations in these error-prone tracts in hMSH3 and hMSH6 are known to occur in cancers showing MSI [12]. This would presumably compound the DNA repair defect. A transition from MSI-L to MSI-H probably occurs in serrated polyps (see below) and adenomas presenting in HNPCC (unpublished findings). However, the MSI-L and MSI-H pathways appear to be divergent rather than sequential in sporadic colorectal cancer [20]. The probability of a transition from MSI-L to MSI-H may also be determined by the nature of the first mismatch repair protein to be rendered non-functioning. For example, germline mutations of hMSH6 lead to an atypical form of HNPCC associated with relatively few colorectal cancers [22]. A reduced probability of conversion from MSI-L to MSI-H may apply when hMSH6 is the first repair gene to be inactivated.

Molecular profiles of colorectal cancers classified according to MSI status

As noted above, mutations in the repetitive sequences of target genes TGFBRII, IGF2R and BAX occur only in colorectal cancers that are MSI-H [3]. Conversely, MSI-H cancers show infrequent LOH, infrequent mutation of APC, K-ras and p53 and retain the normal pattern of β-catenin immunostaining [19, 20]. By contrast, both MSS and MSI-L cancers show frequent mutation of APC, K-ras and p53 and frequent loss of 5q, 17p and 18q [19, 20]. Nevertheless, the overlap of genetic changes in MSS and MSI-L cancers is not perfect. Mutations of APC and K-ras are more common in MSI-L cancers, whereas 5qLOH is more common in MSS cancers [19, 20]. Furthermore, MSS cancers show more BCL-2 overexpression and more aberrant nuclear localisation of β-catenin than MSI-L cancers [20, 21]. While there appears to be an inverse relationship with respect to the establishment of chromosomal versus DNA instability, the two forms of instability may co-exist in a cancer in which the DNA instability is mild (MSI-L). An intriguing property of MSI-H cancers, one that is shared with MSI-L cancers (unpublished observations) but not MSS cancers, is DNA hypermethylation (see below). This further emphasises the intermediate and distinct nature of the MSI-L phenotype. An outline map of a molecular classification of colorectal cancer is shown in Table 1.

Table 1 Molecular profiles of colorectal cancer (CRC) (MMR mismatch repair or mutation in repeat sequence)

<table>
<thead>
<tr>
<th></th>
<th>MSS</th>
<th>MSI-L</th>
<th>MSI-H</th>
<th>HNPCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>++</td>
<td>+++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>MMR mutation</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Allelic loss</td>
<td>+++</td>
<td>++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hypermethylation</td>
<td>–</td>
<td>+++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Percentage of CRC</td>
<td>78%</td>
<td>10%</td>
<td>10%</td>
<td>2%</td>
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