What we could do now: molecular pathology of colorectal cancer

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Abstract
The contribution of molecular genetics to colorectal cancer has been largely restricted to relatively rare inherited tumours and to the detection of germ line mutations predisposing to these cancers. However, much is now known about the somatic events leading to colorectal cancer in general. Several studies have examined the relation between genetic features and prognosis. The purpose of this article is to review these studies and summarise the current state of this subject. Although many of the published studies are small and inconclusive, it is clear that several different pathways exist for the development of this cancer, and some molecular characteristics seem to correlate with clinicopathological features. At present, studies are confined to evaluating a small number of molecular markers; however, with the advent of methods for the rapid genetic profiling of large numbers of colorectal cancers, it will be possible to evaluate fully the clinical usefulness of a range of colorectal cancer genotypes.

Keywords: colorectal cancer; prognosis; genes

Colorectal adenocarcinoma accounts for over 90% of the malignant tumours of the large bowel. After lung and breast cancer, colorectal cancer is the most common cause of death from malignant disease in the Western world. The incidence of the disease in England and Wales is about 30 000 cases/year,1 resulting in approximately 17 000 deaths/annum,2 and it has been estimated that at least half a million cases of colorectal cancer occur each year worldwide.3 Incidence rates of colorectal cancer are increasing in many countries. Unfortunately, despite improvements in medical and surgical provision, there has been comparatively little change in mortality from colorectal cancer during the past 40 years,4 and the overall five year survival is only around 40%.

Natural history of colorectal cancer
In Western countries, approximately 60% of primary colorectal cancers are situated in either the rectum or sigmoid. Of the remainder, a half arise within the caecum. Colorectal cancers are generally staged according to Dukes's system into categories A, B, C, and D.5 Grade can be either expressed simply as degree of differentiation (well, medium, or poorly differentiated) or according to the more complex Jass grouping.6 Prognosis correlates extremely well with both stage and grade.
alternative pathways for the development of cancer that are now known to exist.

INITIATION OF COLORECTAL CARCINOGENESIS

There is good evidence that only two mutations are required for the initiation of colorectal carcinogenesis. In most cases, these mutations occur at the APC tumour suppressor locus (5q21–q22). APC mutations, which generally lead to a truncated APC protein," or take the form of allele loss," are detected in about 75% of sporadic colorectal cancers" and are observed in the earliest adenomas." In addition to the role of the APC gene in the aetiology of sporadic colorectal cancer, germline mutations in this gene cause familial adenomatous polyposis coli (FAP), which is characterised by florid adenomas within the gastrointestinal tract. The APC protein acts as a dimer." It is likely that the gene product exerts its tumour suppressor actions through intracellular signalling, interactions with the cytoskeleton, and the control of cellular proliferation, possibly by affecting the rate of cell division or apoptosis." Whether APC mutations are always the first events in colorectal carcinogenesis or whether germline defects in one of the mismatch repair (MMR) genes could provide an alternative initiating step has been questioned. Mutations in three MMR genes primarily cause the dominantly inherited syndrome hereditary non-polyposis colorectal cancer (HNPPC): MSH2 on chromosome 2p, MLH1 on chromosome 3p, and MSH6 on chromosome 2p.""34 Colorectal cancers from patients with mutations in these MMR genes consistently show microsatellite instability (MSI), a form of replication error (RER). Although it is generally assumed that mutations in the MMR genes in HNPPC families act only to increase the mutation rate (including mutations in APC), it is also possible that the MMR mutations themselves have a direct role in initiation. MMR mutations also occur in sporadic colorectal cancers; however, when these mutations arise somatically they occur after APC mutations and are therefore involved in the progression of tumours rather than initiation.35

Colorectal cancers associated with ulcerative colitis do not usually develop from adenomas, suggesting a different genetic pathway from sporadic cancers. The low frequency of APC mutations in inflammatory bowel disease associated with cancers suggests that mutations in this gene are not the initiating event in these tumour types.

The possibility that mutations in genes other than APC can initiate colorectal tumorigenesis is suggested by reports of adenoma families unlinked to APC. In addition, there is emerging evidence that a hamartoma–adenoma–carcinoma sequence exists, and genes for several hamartoma syndromes have been identified (such as Peutz Jeghers syndrome caused by LKB1 mutations;" some forms of juvenile polyposis caused by SMAD4 mutations," and Cowden’s syndrome caused by PTEN mutations)." Furthermore, there is some support for a hyperplastic polyph adenoma carcinoma sequence (fig 1).39

COLORECTAL CANCER PROGRESSION: EARLY ADENOMA TO CARCINOMA

Although mutations in the APC gene are initiating events in colorectal tumorigenesis, these mutations alone are probably insufficient for adenomas to progress, and without mutations at other loci regression might occur.36 Several genes are involved in the progression of early adenomas to early carcinomas. Early candidates for adenoma progression were the ras oncogenes. The K-ras gene is one of a family of three human ras genes (K-ras, H-ras, and N-ras)." These encode small GTP binding proteins localised to the inner leaflet of the cell membrane and are involved in transducing signals from receptor tyrosine kinases such as epidermal growth factor receptor (EGFR). The receptors are coupled to the ras proteins through an intermediate complex of GRB and SOS2 proteins. Downstream elements of this transduction pathway include the cytoplasmic RAF serine threonine kinase and mitogen activated protein (MAP) kinase cascade. The ras proteins are activated on binding GTP and deactivated by intrinsic GTPase activity from two GTPase activating (GAP) proteins. One of these is ras-GAP-p120 and the other neurofibromin, the product of the NF1 gene. The ras oncogenes are activated by point mutations that prevent the activation of GTPase. More than 50% of colorectal cancers display specific mutations in the K-ras gene, with an increasing frequency in larger and more advanced lesions. The consequence of K-ras mutations during tumour development may be a growth advantage of those cells with both APC and K-ras mutations over cells with APC mutations alone. Whereas K-ras mutations are seen within histologically normal mucosa, they appear only to be present in dysplastic mucosa with coexisting APC mutations. This supports the notion that K-ras mutations confer no growth advantage in the absence of a mutation in the APC gene.

The MCC (mutated in colon cancer) and DCC tumour suppressor genes were originally thought to play a role in colorectal carcinogenesis. In the model of Fearon and Vogelstein, mutation at the DCC locus represented the third step in the genetic pathway. The MCC
and DCC genes were identified as a result of the frequent allele loss close to their locations on 5q21-q22 and 18q21.3, respectively, in colorectal cancers.47 48 A small number of mutations at MCC were originally described in colorectal cancers,49 but subsequent studies have found very few mutations, suggesting that APC is the primary target for allele loss on 5q21-q22.50 DCC is a neural cell adhesion molecule homologue and DCC mutations may therefore have a role in colorectal tumour progression, invasion, and metastasis (although allele loss at DCC generally occurs before malignancy). However, there is evidence suggesting that SMAD4 may also be the target of allele loss on chromosome 18q in some cancers.51

There is no doubt about the role of p53 mutations in the progression of colorectal tumours. The p53 protein is important in maintaining DNA integrity. DNA damage results in p53 mediated arrest in G1 phase of the cell cycle, followed by repair or, if the damage is too great, p53 induced apoptosis. Therefore, loss of function of p53 by mutation or deletion allows cells to accumulate mutations throughout the genome and results in karyotypic instability, impaired G1 cell cycle arrest, and reduced apoptosis.52-55 Mutations in the p53 gene occur in around 75% of colorectal cancers, but the frequency is lower in mucinous cancers and those that arise in the proximal colon, as seen in HNPCC. p53 mutations are rare in adenomas, suggesting that p53 plays a role in tumour progression, but it is not an absolute requirement for malignant transformation because a large proportion of cases have no demonstrable abnormality. p53 mutations tend to occur at the late adenoma stage (although earlier in inflammatory bowel disease associated colorectal cancers, which do not develop from adenomas).56 Dominant gain of function mutations in p53 are common in colorectal cancers, and these can be detected reliably using immunohistochemistry for the p53 protein.57 58 Allele loss near p53 (chromosome 17p13.1) also occurs frequently,59 either because of loss of the wild-type allele or possibly because another gene nearby is targeted.

The sites of other candidate tumour suppressor genes that might be involved in colorectal tumour progression have been identified by allele loss studies. Mutations of the FHIT gene60 and at the p16 (MTS1) locus61-63 may be important in colorectal tumours, the latter through failure of cell cycle arrest. Locations of other tumour suppressor loci include chromosomes 1p (near the putative human Mom-1 homologue),64 65 66 8p,67 14q,68 and 22q.69 Allele loss typically occurs at these locations at frequencies of between 30% and 60%.

The roles of MMR mutations in colorectal tumorigenesis have been discussed briefly. In HNPCC tumours, one mutation is inherited and the other occurs somatically; in about 15% of sporadic colon cancers, two MMR mutations (or two mutations at a related locus) occur in the soma.70 Normal mucosa from patients with HNPCC does not display MSI; only 50% of HNPCC adenomas (compared with 90% of cancers) exhibit MSI, and the frequency of early lesions such as APC mutations is similar in MSI and MSS (microsatellite stable) tumours.71 72 Therefore, in sporadic cancers, defective MMR function might be an alternative method to allele loss of acquiring mutations, and loss of MMR may simply “catalyse” the progress of a tumour down the same pathway as MSS cases. It is possible that MMR mutations act as alternatives to p53 mutations in colorectal tumours, albeit through a different mechanism. Whereas genomic instability in p53 mutant cancers tends to take the form of karyotypic abnormalities, instability resulting from MMR mutations leads to near diploid lesions. It has been found that MMR mutations are negatively associated with mutant p53 and that, like p53 mutations, MMR mutations often occur in late colonic adenomas.72 73 Genomic instability may also be caused by somatic or germline mutations in other genes involved in DNA replication and repair. There is support for this from a study of two apparently sporadic colorectal cancer cases that harboured DNA polymerase δ variants.74

MALIGNANCY

Few genetic changes specific to mature colorectal cancers have been identified. Several regions of allele loss have been detected, but their roles in tumour progression are unclear. The types of mutations that will be important in mature colorectal cancer are those that cause faster replication, decreased apoptosis, or angiogenesis. Malignancy requires colorectal tumour cells to exhibit several features, namely: (1) the ability to erode the basement membrane; (2) the ability to disrupt normal cell junctions; and (3) the capacity to survive in the blood or lymphatic systems and in a new tissue environment.

Cell adhesion molecules are candidates for involvement in the process of invasion and metastasis of colorectal cancers. Mutations at these loci may have effects on growth, in addition to those on adhesion. For example, E cadherin forms part of the adherens junction complex of epithelial cells. Loss of E cadherin protein occurs in several cancers, including colorectal cancer, and is associated with the development of invasive properties.75-77 Other proteins that may be associated with invasion of colorectal cancers include those involved in tissue degradation, such as urokinase plasminogen activators and matrix metalloproteinases/collagenases.78 79 The cells of metastases may have genotypes and/or patterns of gene expression distinct from primary tumours. Variation at several gene loci may alter the behaviour pattern of the mature colorectal cancer. These include the NM23 gene, which has a possible role in the metastasis of several cancers, and CD44.80-85

Clinicopathological correlations: prediction of prognosis

The recognition that there are probably several different genetic pathways for colorectal cancer suggests that correlations exist between the
molecular and clinicopathological features of tumours that are not apparent using routine methods such as histology. These correlations may serve as prognostic determinants and/or enable the partitioning of patients with colorectal cancer into groups for different treatments. Several studies have sought to examine the relation between genotypic variation in colorectal cancers and clinicopathological features, especially prognosis. Most work has been in the form of case control studies using a comparison of the frequency of genotypes in primary and secondary tumours as a surrogate for survival. However, a small number of studies have examined the relation between genotype and prognosis by classic survival analysis. In this section, the evidence for genetic variation in colorectal cancers as markers of prognosis is reviewed, including some studies of protein or mRNA expression that have been assumed to be indicators of underlying mutations.

**ALLELE LOSS STUDIES**
In an early allelotyping study based upon 56 patients with colorectal cancer, Vogelstein *et al.* showed that patients with more than the median percentage of allelic deletions had a worse prognosis. Later studies have examined the relation between specific chromosome abnormalities and tumour behaviour. Chromosome 18q loss has been evaluated extensively as a prognostic marker in several studies. In a study of 145 patients with resected stage II or stage III disease reported by Jen *et al.*, the five year survival rate was 93% in those with no loss and 54% in those with chromosome 18q loss in stage II disease, and 52% and 38%, respectively, with stage III disease. The overall estimated hazard ratio (HR) for death associated with tumour chromosome 18q loss was 2.8 (p < 0.01) in a univariate analysis. Allelic loss remained a strong predictive factor after adjustment for tumour differentiation, vein invasion, and TNM stage (HR = 2.46; 95% confidence interval (CI), 1.06 to 5.71; p = 0.04). A similar finding was reported by Ogunbiyi *et al.* (HR = 2.46; 95% CI, 0.27 to 5.10). The high rate of allele loss in other chromosomes such as 8p, 1p, and 11q during tumour progression suggests that these may be sites of other tumour suppressor genes important for the progression of colon tumours. A correlation between 8p allele loss and microinvasion (a prognostic marker independent of Dukes’s stage) has been reported in one small study of 14 patients with cancer. A relation between tumour progression and chromosome 18q deletions has also been shown in one study of 116 patients who had undergone curative treatment (HR = 4.1; 95% CI, 1.25 to 9.23).

Allele loss at other chromosomes has been evaluated less extensively. An analysis of 126 sporadic colorectal cancers for allele loss at chromosome 11q22 failed to show a relation with Dukes’s grade or degree of differentiation. A prospective study assessing allelic loss at chromosome 4p14–16 in 181 patients has suggested that allele loss may be associated with a shorter disease free survival.

**K-RAS**
Along with p53, K-ras mutations are one of the most common genetic lesions in human cancer. Since the discovery of the human ras gene family, the effect of different ras mutations on tumour behaviour have been debated. Point mutations in codons 12, 13, and 61 of the K-ras gene are early events in the pathogenesis of colorectal cancer. However, the impact of the number, type, and position of such mutations on the progression of adenomas, in addition to the clinical behaviour of colorectal carcinomas, is not fully established. To date, most studies have indicated that the second base of codon 12 is more heavily mutated in colorectal cancer than the first or the third bases. Therefore, it is conceivable that the type of K-ras mutation directly determines tumour behaviour.

In a study of 194 consecutive primary, recurrent, and metastatic colorectal adenocarcinomas, Finkelstein *et al.* reported a significantly higher mutation rate of K-ras mutations in lymphogenous haematogenous metastatic disease. When colorectal carcinomas were analysed by specific K-ras mutation type, codon 13 mutated tumours did not progress locally or metastasise. Tumours with a codon

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12 valine substitution did not metastasise beyond pericolonic perirectal lymph nodes. In contrast, codon 12 aspartic acid substitutions were common in cancers exhibiting distant deposits. No mutations were common in tumours with intraperitoneal deposits. On the basis of these data, Finkelstein et al proposed that genotyping of colorectal adenocarcinoma for K-ras status would identify subsets of patients likely to have indolent or aggressive forms of disease.10 Some, but not all, reports have supported the proposal that the possession of a K-ras mutation is independently associated with shorter survival.102–106 To clarify the association between K-ras mutations, patient outcome, and tumour characteristics a meta-analysis of data from 22 research groups (total number of cases 2721) was undertaken by Andreyev et al.107 Mutations were not associated with sex, age, site, or stage. Poorly differentiated tumours were less frequently mutated (p = 0.002). Multivariate analysis suggested that the presence of a mutation increased risk of recurrence (p < 0.001) and death (p = 0.004). In particular, any mutation of guanine to thymine but not to adenine or to cytosine increased the risk of recurrence (p = 0.006) and death (p < 0.001). When specific mutations were evaluated, only the codon 12 valine mutation was found to convey an independent, increased risk of recurrence (p = 0.007) and death (p = 0.004). The biological basis of a relation between a specific K-ras mutation and tumour behaviour is not straightforward. Early in vitro observations suggested that codon 12 K-ras mutations elicited stronger transformation responses than codon 13 K-ras changes in NIH/3T3 cell assays.108–110 However, this notion has not been supported by biochemical analyses of different ras proteins.111–113 On the basis of the available clinicopathological data, it is likely that patient survival is related to the occurrence of K-ras mutations, but not necessarily to the specific type of mutation.

DCC

If allele loss of chromosome 18q predicts a poor outcome in colorectal cancer, then the DCC gene must represent a prime candidate as the cause for this association. In a study of 132 patients with curatively resected stage II and III carcinomas, the expression of the DCC protein has been shown to be a strong positive predictive factor for survival.114 Patients with stage II disease whose tumours expressed DCC had a five year survival rate of 94%, compared with a survival rate of 62% for patients with DCC negative tumours. Similarly, in individuals with stage III disease, the respective survival rates were 59% and 33% in those with and without detectable DCC expression. This finding has been supported by a recently published small study of 23 patients.115

Mismatch Repair Genes and Microsatellite Instability

Evidence directly correlating molecular and clinicopathological data for colorectal cancer has come from HNPCC. Colorectal cancers developing in carriers of HNPCC mutations are poorly differentiated and frequently multiple. Paradoxically, despite multiplicity and poor differentiation of cancers, early observations suggested that colorectal cancers in HNPCC carried a more favourable prognosis that in sporadic cases. Convincing evidence for improved survival in HNPCC is provided by studies from Finland116 and Japan.117 Whether MSI characterises a subset of sporadic colorectal cancers with a different prognosis has been evaluated in several studies. One of the early studies reported by Bubb et al in 1996 was based on a systematic analysis of 215 sporadic cases.118 Patients with MSI tumours had a significant survival advantage over those with MSS tumours, independent of other prognostic factors (HR = 0.39; 95% CI, 0.19 to 0.82). Several subsequent studies have reinforced this finding. In a study of 66 cases, Massa et al found that MSI status was independently associated with an improved prognosis (defined by overall survival and disease free survival).119 A large study based on 607 patients reported by Gryfe et al also found that MSI status conferred a significant survival advantage, independent of all standard prognostic factors, including tumour stage (HR = 0.42; 95% CI, 0.27 to 0.67). Regardless of the depth of tumour invasion, colorectal cancers with MSI had a decreased likelihood of metastasising to regional lymph nodes (HR = 0.33; 95% CI, 0.21 to 0.53) or distant organs (HR = 0.49; 95%, 0.29 to 0.89). A similar relation was seen between MSI status and prognosis in a study of 197 patients reported by Johannsdottir et al.120 Although MSI was not associated with clinicopathological parameters, such as Dukes's stage and tumour differentiation, this phenotype was associated with better overall survival. From an analysis of 225 colorectal cancer cases, Jernvall et al found that the five year survival rate in MSI cancers of the proximal colon was much better (100%) than that of those with MSS proximal disease (74%).121 However, an opposite effect was seen in relation to distal disease. In a study of a consecutive series of 388 Dukes's C colon carcinomas with five year median follow up reported by Elsleith et al,122 the presence of MSI in the proximal transverse colon carcinoma group was associated with a significantly better prognosis (58% v 32%; p = 0.015). This was largely the result of the better survival seen in the MSI subgroup who received adjuvant chemotherapy, leading to the proposal that MSI status might determine the response to adjuvant chemotherapy. However, this seems unlikely to be the sole explanation of a better prognosis. Wright et al found MSI status conferred a significant survival advantage in patients who had not been in receipt of chemotherapy.123 Furthermore, Hemminki et al found that adjuvant 5-FU based chemotherapy is feasible both for patients with MSI and those with MSS.124 The three year recurrence free survivals were 90% and 43% in the MSI and MSS groups, respectively. Although most studies have found a relation between MSI status and prognosis some have not, including a report by
Salashor et al., based on a survival analysis at five to 10 years follow up of 181 unselected colorectal cases. Given that only approximately 15% of cancers display MSI, failure to demonstrate a difference might be the result of a lack of power in this study.

Overall, the weight of evidence supports the tenet that although the genetic basis of HNPPC and sporadic cancers with MSI is different, tumours in the two groups share some biological characteristics in terms of prognosis. MSI in colorectal cancer appears to be an independent predictor of a relatively favourable outcome and, in addition, reduces the likelihood of metastases.

p53 AND p27

An increased intracellular concentration of p53, which is frequently but not systematically related to p53 mutation, has been proposed to be associated with poor prognosis in some tumour types. In colorectal cancer, although p53 overexpression in tumours correlates with chromosome 17p loss, hyperdiploid DNA content, and tumour site, there have been conflicting findings about its role as a prognostic indicator in studies based on immunohistochemistry. This reflects the fact that the degree of association between p53 mutations and protein expression depends in part on the specific antibody used. In studies of the relationship between p53 mutations and prognosis, the situation is clearer. Studies suggest that colorectal cancers harbouring p53 mutations are more aggressive, are associated with a higher propensity for lymphatic and haematogenous spread, and have a worse prognosis. However, this is not universal.

p27 is a member of the cip/kip family of cyclin-dependent kinase inhibitors, which bind to cyclin/cyclin-dependent kinase (cdk) complexes and block progression through the cell cycle. p27 regulates progression from G1 into S phase by binding to and inhibiting the cyclin-E/Cdk2 complex, which is required for cells to enter S phase. In contrast to the p53 gene, mutations in p27 are rare. However, cell cycle regulation of p27 concentrations are regulated at the post transcriptional level through proteasome mediated degradation. Reduced expression of p27 was first shown to correlate with poor survival in a study of 149 patients reported by Lodha et al. Patients whose tumours expressed p27 had a median survival of 151 months whereas those that lacked p27 (10%) had a median survival of only 69 months. In the study, p27 expression was reported to be an independent prognostic marker and the risk of death associated with reduced expression was increased 2.9-fold. Subsequent studies have confirmed that p27 appears to have prognostic importance. Furthermore, decreased p27 expression has been associated with an increased likelihood of lymph node metastases in colon cancers, independent of depth of tumour invasion.

Conclusions

The incidence of colorectal cancer is increasing for most patients. The identification of those patients who are at a high risk of recurrent local and metastatic disease is important in selecting the appropriate treatment. Prognostic variables that have been found to have a significant effect include pathological stage and grade, type of tumour growth, chromosomal aneuploidy, and the presence of microinvasion. Until recently, there has been little understanding of the molecular basis of these indices. Despite the continuing use of histopathology as the "gold standard", the genetic features of colorectal tumours will almost certainly become useful indicators of prognosis and of the most appropriate treatment. One of the problems with clinical-molecular associations reported to date has been that most studies have, for entirely understandable reasons, analysed only small numbers of tumours. Publication bias is a serious problem in assessing the usefulness of any given marker because positive associations have a far higher probability of being published than negative ones. Contradictory results between studies may reflect in part heterogeneity in colorectal cancers. It has been suggested that multiple biopsies and DNA sampling preceded by careful morphological examination must be standard if the preparation of DNA is required.

Accepting these caveats, there is evidence that colorectal cancers harbouring defects in the MMR genes are associated with a better prognosis and those with chromosome anomalies, such as chromosome 18q or 17 deletions and mutations in K-ras and p53, with a worse prognosis. With the advent of methods for rapid genotyping it should be possible to construct mutation profiles of tumours and use multivariate analysis to determine which molecular features correlate with the clinicopathological data.


Colorectal cancer: what could we do now?


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