Review Article

Colorectal Cancer: Molecular Features and Clinical Opportunities

***Daniel L Worthley,[1,2] Barbara A Leggett[1,2]**
[1]Conjoint Gastroenterology Laboratory, Royal Brisbane and Women's Hospital Research Foundation Clinical Research Centre, Brisbane, QLD, Australia
[2]Queensland Institute of Medical Research, Brisbane, Qld, Australia.
*For correspondence: Dr Daniel Worthley daniel.worthley@uqconnect.edu.au

Abstract
Colorectal cancer is a heterogeneous disease. There are three main pathways to colorectal cancer: the chromosomal instability pathway, the CpG island methylator phenotype pathway and the pure microsatellite instability pathway. Each of these is characterised by specific pathological precursors, mechanisms of carcinogenesis and natural history. The molecular features of these pathways have been exploited clinically in the diagnosis, screening and management of patients and families with colorectal cancer. This review summarises recent developments in our understanding of colorectal carcinogenesis and examines the interface between scientific discovery and the clinical application of molecular techniques in inherited and sporadic colorectal cancer.

Introduction
Colorectal cancer (CRC) is the second most frequently diagnosed internal malignancy in Australia and the second most common cause of cancer death.[1] In 2005, over 13,000 Australians were diagnosed with, and over 4000 Australians died from, CRC.[1,2] These are sobering statistics for a cancer with well-defined risk factors originating, in the vast majority of cases, from a slowly progressive precursor lesion within reach of, and cured by, colonoscopic polypectomy. Furthermore, surgical resection of stage I CRC is associated with 90% long term survival.[3]

CRC, however, is a heterogeneous disease. CRCs and their pathological precursors display distinct molecular signatures, distinct pathological features and distinct natural histories. There are at least three major molecular pathways to CRC including the predominant chromosomal instability (CIN) pathway accounting for up to 85% of cases, the CpG island methylator phenotype (CIMP) pathway which is the other major pathway to sporadic CRC[4] and includes sporadic microsatellite instability (MSI) high cancers and finally the pure MSI pathway resulting from germline mutation in a DNA mismatch repair (MMR) gene. Hereditary non-polyposis colorectal cancer (HNPCC) develops via the pure MSI pathway.

There is currently a multitiered approach to reducing the burden of CRC through both the earlier diagnosis of cancer and the detection and removal of benign polyp precursors. These clinical strategies have been informed by our understanding of the molecular pathology underpinning colorectal carcinogenesis. For the vast majority of the population that are asymptomatic and without any significant family history of CRC, screening is by an average risk strategy, such as faecal occult blood testing in Australia.[2] Symptomatic patients, or those with a past history of colorectal neoplasia are subjected to diagnostic and surveillance investigations usually colonoscopy. Patients likely to have familial CRC syndromes are identified and managed by combined endoscopic and molecular testing.

This review explores the molecular basis of colorectal carcinogenesis and touches on the potential clinical opportunities for molecular screening and diagnostic testing in the future.

Colorectal Cancer: the Pathways
CRCs are heterogeneous in terms of molecular momentum, regional distribution, pathology of the invasive and precursor lesions and natural history (Figure).[5]

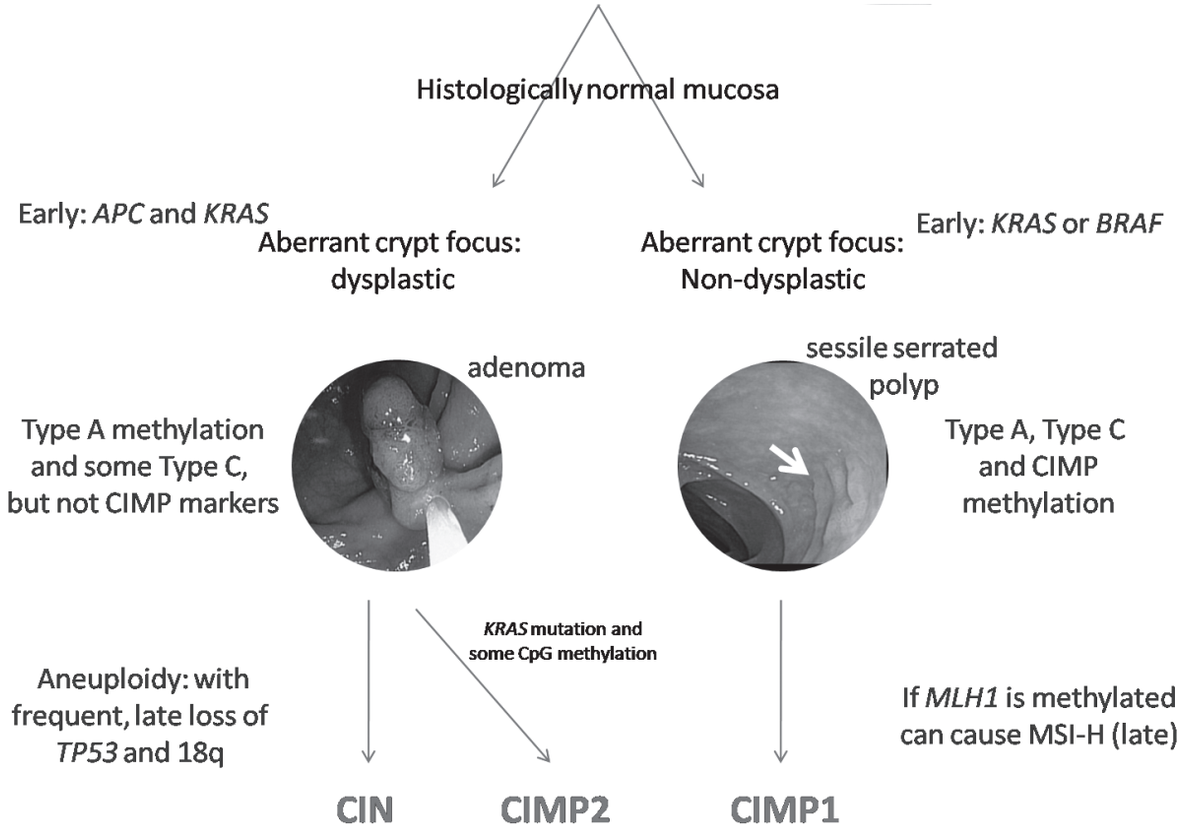

Figure. A simplified working-model of sporadic colorectal carcinogenesis: the CIN and CIMP pathways. In 'Type A' genes considerable promoter methylation may be observed even in normal tissue and is associated with tissue ageing. 'Type C' marker promoter methylation is more specific for neoplastic tissue.

Regardless of the underlying pathway, for a cancer to develop multiple and sequential genetic alterations must occur. Each genetic perturbation establishes a successive clone, with a 'successful cancer' requiring up to 10 clonal events, each characterised by relative growth advantage.[6] These pre-cancerous cells must also develop a cellular environment permissive of future genetic and possibly epigenetic events, that is genomic and epigenomic instability, respectively.[7] Genomic instability is critical in carcinogenesis to ensure that the subsequent events occur at increasingly greater likelihood. It accelerates the neoplastic evolutionary process by increasing the mutation rate induced by the background mutagenic challenge. Without genomic instability, the acquisition of new mutations would occur far too slowly for a cancer to develop during a person's lifetime.[8] As mentioned above, there are at least three main molecular and pathological pathways to CRC - the CIN, the CIMP and the pure MSI pathways.

The Chromosomal Instability (CIN) Pathway

Approximately 70%-85% of CRCs develop via the CIN pathway.[9] In the CIN pathway molecular aberrations occur in significant part through the accumulation of numerical or structural chromosomal abnormalities (aneuploidy).[6] The earliest identifiable lesion in this pathway is the dysplastic aberrant crypt focus (ACF),[10] a microscopic mucosal lesion that precedes the development of a polyp.[11] The CIN pathway is associated with mutation in *APC* and/or loss of chromosome 5q that includes the *APC* gene, mutation of the *KRAS* oncogene, loss of chromosome 18q and deletion of chromosome 17p, which contains the important tumour suppressor gene *TP53*.[9] Only a very small minority of CRCs characterised by CIN, however, possess a full complement of these molecular abnormalities.[12] It is possible that several of these clonal events can be bypassed by other genetic or epigenetic aberrations in order to deliver the necessary biological consequences.[13] Sequencing the human genome has provided the opportunity for more detailed analysis of tumour-specific mutations with many potentially important genetic events now identified outside of the genes referred to above.[14] Many of these will ultimately be shown to be additional, alternative, and perhaps complementary steps to these traditional genes within the adenoma-carcinoma sequence.

APC is an extremely important tumour-suppressor gene in the CIN pathway to CRC. Pathogenic mutations in *APC* frequently truncate the APC protein and interrupt the binding of APC to β-catenin. The binding of APC to β-catenin helps to suppress the *Wnt*-signaling pathway.[15] *Wnt* signalling regulates growth, apoptosis and differentiation and is particularly relevant in maintaining tissue specific stem cell compartments.[16] Loss of functional APC might also interfere with the careful regulation of mitosis contributing to CIN.[17] The frequency of *APC* or *β-catenin* mutation in early adenomas has been reported to be as high as as 80%,[11] although the rate of *APC* mutation is significantly lower in some series.[18] Mutation of *APC* is found in approximately 60% of colonic and 82% of rectal cancers.[18]

KRAS (12p12) is another important gene within the CIN pathway. *KRAS* encodes a GTP-binding protein which, when mutated, can cause a loss of inherent GTPase activity and thus constitutive signalling through the downstream, RAS-RAF-MEK-ERK pathway.[19] BRAF is another important factor in this signalling cascade particularly relevant in the CIMP pathway, which is discussed below. Activating *KRAS* mutations are found in 35-42% of CRCs and in a similar number of advanced adenomas.[19,20] The role of *KRAS* is not unique to the CIN pathway, however, and *KRAS* has an important role in the CIMP pathway as well.[21]

DCC, SMAD2 and *SMAD4* are all located at 18q21.1 and allelic loss at this site is found in up to 60% of CRCs.[22] *SMAD2* and *SMAD4* are involved in the TGF-β signalling pathway, which is important in regulating growth as well as apoptosis. Germline mutation of *SMAD4* can cause generalised juvenile polyposis syndrome, which is associated with CRC.[23]

Finally, impairment of *TP53* (17p13) usually through allelic loss of 17p is often a late event in the traditional pathway accompanying the transition from adenoma to adenocarcinoma. *TP53* abnormalities, either mutation or loss of heterozygosity, increase relative to the advancing histological stage of the lesion being studied, with 4-26% of adenomas, 50% of adenomas with invasive foci, and 50-75% of CRCs having impaired function of *TP53*.[19] The p53 protein normally acts to increase the expression of cell-cycle genes, to slow the cell cycle and provide sufficient time for DNA repair. Furthermore, when the genetic damage sustained is too great for the cell to repair, p53 induces pro-apoptotic genes, thus containing the genetic insult through programmed cell death.[24]

The CIN pathway and its accompanying pathological adenoma-carcinoma sequence has provided a foundation for the molecular classification of colorectal carcinogenesis and has established a reference against which one may contrast other CRC molecular profiles, but it is now clear that CRC may develop by other means.

The Microsatellite Instability (MSI) Pathway

Another important type of genomic instability is MSI. Microsatellites are nucleotide repeat sequences scattered throughout the genome and MSI refers to a discrepancy, and thus instability, in the number of nucleotide repeats found within these microsatellite regions in tumour versus germline DNA. DNA polymerase is particularly susceptible to making errors when copying these short repeat sequences and thus mismatch repair (MMR) dysfunction results in MSI. The MMR system is composed of at least seven proteins, MLH1, MLH3, MSH2, MSH3, MSH6, PMS1 and PMS2, which associate with specific partners to form functional heterodimers.[7] MLH1 and MSH2 are essential in the mismatch repair machinery and form five functional heterodimeric proteins (MSH2-MSH3; MSH2-MSH6; MLH1-PMS1; MLH1-PMS2; MLH1-MLH3). Mutations in *MLH1, MSH2, MSH6* and *PMS2,* have all been implicated in HNPCC. Many CRCs with an intact MMR system, however, will have frameshift mutations at a small number of microsatellites and thus a standardised panel of microsatellites was devised to provide uniformity of definition for research and practice.[25] The currently endorsed panel includes two mononucleotide (BAT25 and BAT26) and three dinucleotide microsatellites (D5S346, D2S123, and D17S250). Considerable MSI or MSI-high (MSI-H) is defined as MSI at ≥2 (40%) of the five specified sites, MSI-low (MSI-l) as MSI at one site, and microsatellite stable (MSS) when no instability is demonstrated at these markers. MSI leads to a dramatic increase in genetic errors and several microsatellites are present in genes implicated in colorectal carcinogenesis, such as *MSH3, TGFBR2, BAX, CASP5, MSH6, CTNNB1, APC, IGF2,* and *E2F4*.[26] MSI-H cancers are usually diploid, as MMR dysfunction provides the mechanism of genomic instability without the biological imperative for concomitant CIN.

Whilst HNPCC causes the pure form of MSI, the majority of MSI-H CRCs occur sporadically in the context of DNA methylation of the *MLH1* promoter and the consequent transcriptional silencing of *MLH1* expression. Such cancers exhibit both CIMP and MSI, and are considered in this article as part of the CIMP pathway. MSI-H tumours, whether sporadic or inherited, however, share similar biology.

The CpG Island Methylator Phenotype (CIMP) Pathway

The CIMP pathway is the second most common pathway to sporadic CRCs. The CIMP pathway accounts for approximately 15% of sporadic cases.[4,27-30] The CIMP pathway provides the epigenetic instability necessary for sporadic cancers to

methylate the promoter regions of, and thus epigenetically inactivate the expression of, key tumour suppressor genes such as *MLH1*. CIMP-positive CRCs are currently defined by a panel of CpG island methylation markers, that are classified as having or not having DNA methylation on the basis of certain thresholds. The CIMP panel of genes and or markers is analogous to the panel of microsatellites used to determine microsatellite status.[27] There is not yet, however, one universally endorsed panel of CIMP markers or even a gold-standard technique for characterising methylation for the diagnosis of CIMP. There has been a great deal of research into the best markers and in our laboratory we have adopted a five marker panel *CACNA1G, IGF2, NEUROG1, RUNX3,* and *SOCS1*, examined by a methylation specific, probe-based real-time PCR technique called *MethyLight.*[27] In this panel, methylation is defined quantitatively and cancers are characterised according to their percent of methylated reference (PMR). Cancers with a PMR of ≥10 at three or more of these gene promoter sites are classified as CIMP positive.[27] Using this panel, there is a strong association between CIMP-positive cancers and *BRAF* mutation.[27] Some investigators, however, rightly advocate for the further refinement of the CIMP-positive group into CIMP-low (or CIMP2) and CIMP-high (or CIMP1) categories.[21] A three-tiered system probably provides a more biologically consistent means of classification, with CIMP2 CRCs showing a closer association with *KRAS* rather than *BRAF* mutation, and there is the suggestion that CIMP2 CRCs may even develop from unique pathological precursors, possibly tubulovillous adenomas.[21,30] Dissecting out these detailed pathways is an area of intensive ongoing research.

CIMP-positive CRCs are characterised by a well defined cluster of clinicopathological features, including proximal location and a gender and age bias for the development of CIMP in older women.[28-30] The ultimate phenotype is influenced by the presence or absence of concomitant microsatellite instability, which may arise from gene promoter methylation-induced transcriptional silencing of *MLH1*. Classically, the CIMP-positive CRCs that are MSI-H share MSI-H characteristics, specifically the relative good prognosis, but in the absence of MSI-H, the CIMP-positive phenotype is characterised by more advanced pathology, poorer clinical outcome and an absence of tumour-infiltrating lymphocytes.[30] Very importantly, CIMP-positive CRCs differ from the other pathways with respect to their precursor lesion.[28-30] CRCs developing via the CIN pathway, and also in HNPCC, originate from adenomatous polyps.[31,32] In the CIMP pathway, however, sessile serrated adenomas are the chief pathological precursor.

Colorectal Polyps: Pathway Precursors

Adenomatous polyps with their well-recognised pathological progression from simple to advanced adenoma and ultimately invasive adenocarcinoma are the established precursors to the CIN pathway as well as HNPCC cancers.[18,33-35] As noted above, the serrated polyp precursors to the CIMP pathway are now also recognised as important polyps, although knowledge about the exact pathological sequence of serrated neoplasia is still evolving.[18,30,34,36,37] These CIMP-precursor polyps are referred to as 'serrated' in order to describe the sawtooth appearance of their crypt lumina.[37] Their serrated crypt columns are generated due to an underlying defect in apoptosis shared by these polyps, in turn, the consequence of overactive RAS-RAF-MEK-ERK signalling. This leads to a build-up of colonocytes and thus the sawtooth appearance.[37]

The earliest histopathology within the CIMP pathway may be the serrated, non-dysplastic ACF. There is some evidence, however, that there may be disturbed DNA methylation even within the apparently "normal" colorectal mucosa.[38,39] Therefore, these lesions may develop in the context of a primed or predisposed epithelial field. These early non-dysplastic ACFs probably evolve into simple hyperplastic polyps, further characterised as mucin-poor, goblet-cell-rich or microvesicular. The goblet-cell-rich hyperplastic polyp is the typically diminutive rectosigmoid hyperplastic polyp, a common finding at colonoscopy and believed to have negligible malignant potential. In contrast, it is the microvesicular hyperplastic polyp that shows a high rate of *BRAF* mutation and thus is the most likely candidate to be the polypoid precursor to the CIMP pathway. *BRAF* mutation and CIMP marker methylation are seldom found in tubular adenomas.[40-44] Higher CIMP levels (methylation at four or more CIMP markers), in particular, are specific for the serrated pathway precursors, with approximately 30% of advanced serrated polyps being positive compared to 0% of advanced adenomas.[40] Sessile serrated adenomas, which are a more advanced polyp, are characterised by increased serration of the surface and luminal crypt epithelium, dilation of the crypt base, horizontal crypt branching, increased intracellular and/or luminal mucin, cytological atypia in the upper crypt, enlarged vesicular nuclei with prominent nucleoli and an increased epithelial to stromal cell ratio.[36] The pathological progression of serrated neoplasia may then include mixed polyps and traditional serrated adenomas. In the traditional serrated adenomas the dysplasia is an integrated component of the serrated polyp, whilst in the mixed polyp, the dysplasia exists as a discrete element, separate to the serrated architecture. All of the serrated polyps are likely to be related, but whether each represents a different stage of a uniform and sequential pathological process is still uncertain. Many of these polyps, however, share the common *BRAF* mutation and other characteristics of the serrated neoplasia pathway.[44] The serrated morphology of the precursor polyps is often lost

following malignant transformation into the CIMP-positive cancer. Nevertheless, whilst all CIMP-positive cancers are not serrated adenocarcinomas, those that are share many molecular features with their serrated precursors.[45]

Translating Discoveries into Practice

There are many examples of CRC pathology informing practice, particularly in familial CRC syndromes. The bench-to-bedside approach in such families has established colorectal medicine at the forefront of translational research and these families have greatly informed our understanding of CRC overall. Below we review some of the clinical circumstances in which molecular colorectal carcinogenesis research has informed our current practice.

Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome characterised by >100 colorectal adenomas. Without surgical intervention, CRC occurs in essentially all cases of FAP by the age of 50 years.[2,46] FAP is due to a pathogenic mutation in *APC,* a very important gene in the CIN pathway, as described above. Interestingly, the site of mutation within *APC* influences the resultant phenotype both in terms of severity as well as the associated extracolonic features.[46] Mutations within the central region of the *APC* gene are generally associated with a higher colorectal burden of adenomas, whilst mutations at the 5′ or 3′ ends of the gene usually result in a milder phenotype, so-called attenuated FAP (AFAP). AFAP is characterised by <100 colorectal adenomas, and the development of CRC is delayed by approximately 15 years compared to classical FAP.[46] Another inherited polyposis syndrome, *MUTYH*-associated polyposis (MAP) is an autosomal recessive cause of multiple colorectal adenomas and cancer. MAP is often similar in phenotype to AFAP.[47,48] In contrast to FAP, MAP is primarily a DNA repair disorder. MUTYH is a DNA glycosylase which helps to repair mispaired bases that develop following oxidative DNA damage. MUTYH protects against mutations in genes such as *APC* and *KRAS,* critical in the CIN pathway to CRC.[46,48] Bi-allelic mutations in *MUTYH* confer a 93-fold increased risk of CRC.[49,50] Although local recommendations vary, clinical genetics referral, counselling and testing for germline *APC* and *MUTYH* mutations should be considered in patients with 20 or more colorectal adenomas (including metachronous lesions) or in patients younger than 60 years with >/= 5 adenomas and either a personal history of, or a first- or second-degree relative with, CRC or adenoma with high-grade dysplasia also before 60 years. The sequence of testing is often directed by the family history.

Several clinicopathological criteria have been developed to identify patients and families that are at higher risk of HNPCC. These criteria help to identify at-risk tumours that should undergo further molecular testing often by MSI analysis and immunohistochemistry for the four MMR proteins implicated in HNPCC i.e. MLH1, MSH2, MSH6 and PMS2 (see Table).[2,51-54] If the index CRC is MSI-H or has selective loss of protein expression in the tumour, then germline testing of the proband is organised. The first gene to analyse is directed by the immunohistochemistry results. It is important to appreciate, however, that loss of one member of a mismatch repair heterodimer pair, as described above, can lead to loss of the complementary member of the heterodimer. For instance *MLH1* mutation can lead to the loss of both MLH1 and PMS2 staining on tumour immunohistochemistry.

BRAF is not mutated in HNPCC cases and thus there is an emerging role for *BRAF* testing in MSI-H cancers, particularly when wishing to confirm that the cancer developed via the

Table. The Revised Bethesda Guidelines for further testing of colorectal tumours, such as immunohistochemistry for *MLH1, MSH2, MSH6* and *PMS2* and microsatellite instability.[53]

Tumours should undergo further molecular testing in the following situations:
Colorectal cancer diagnosed in a patient who is less than 50 years of age.
Presence of synchronous, metachronous colorectal, or other HNPCC-associated tumours,* regardless of age.
Colorectal cancer with the MSI-H** histology*** diagnosed in a patient who is less than 60 years of age.
Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed under age 50 years.
Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumours, regardless of age.

*HNPCC-related tumours include colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumours, sebaceous gland adenomas and keratoacanthomas in Muir–Torre syndrome and carcinoma of the small bowel.
**MSI-H refers to changes in two or more of the five National Cancer Institute-recommended microsatellite markers.
***Presence of tumour infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

sporadic CIMP pathway and thus exclude HNPCC.[28,55] *BRAF* testing of the tumour may be helpful for clarifying the relevance of MSI-H cancers diagnosed in older patients, in whom MSI-H cancers are more likely to have resulted from CIMP related methylation of *MLH1* rather than HNPCC.[28,54] It is important to note that not all HNPCC adenoma precursors will demonstrate abnormal immunohistochemistry or MSI as these are both relatively late events in the HNPCC adenoma-carcinoma sequence.[56] Using a combination of immunohistochemistry and MSI testing may miss up to a quarter of HNPCC adenomas, albeit that it is of course clinically very helpful when an abnormality is found.[56] Finally, it is important to note that specific MMR gene mutations are associated with differing clinical phenotypes. For instance, patients and families with germline mutations of *PMS2* and *MSH6* have a lower penetrance of CRC than those with mutations of either *MLH1* or *MSH2*.[57,58]

Beyond these familial examples, molecular analysis of CRC is also now mainstream in planning the management of advanced CRC. The monoclonal antibodies cetuximab and panitumumab, which target the epidermal growth factor receptor (EGFR) and thus the RAS-RAF-MEK-ERK pathway, have been an important development in the management of metastatic CRC. It became clear, however, that some patients were resistant to the clinical benefits of these agents. Patients with *KRAS* mutant cancers or those with wild-type *KRAS* but mutations in *BRAF* or *PIK3CA* are less likely to respond to these EGFR-directed therapies.[59] Cancers are now routinely tested for *KRAS* mutations prior to starting these therapies.

There is burgeoning interest in the application of molecular techniques for the screening and surveillance of patients at average risk of CRC. Serum technologies have been explored, including the testing for circulating tumour DNA encoding detectable mutations in *APC, TP53* and *KRAS*.[60,61] Currently it is the molecular analysis of stool-based mutations, however, that may hold the greatest promise.[62-65] The multiple pathways to cancer, however, mandate either a very broad, inclusive marker panel or alternatively several specific panels each designed for use in distinct clinical circumstances. Molecular CRC screening in average risk people promises to develop into an important strategy in the detection of advanced colorectal neoplasia, which will ultimately be necessary for the prevention of CRC.

Summary

The discussion above outlined the three main pathways to CRC: the CIN, the CIMP and the pure MSI pathways. The CIN pathway is characterised by aneuploidy and is associated with the stepwise mutation or loss of *APC, KRAS, SMAD2, SMAD4* and *DCC,* and *TP53*. The *Wnt*-signalling pathway is important in many of these cancers. The RAS-RAF-MEK-ERK pathway is another important pathway in CRC, in both the CIN and CIMP pathways. The CIN pathway passes through simple and then advanced adenomatous stages and, in the minority that develop into invasive disease, an MSS cancer. A minority of cancers, less than 5%, develop via the pure MSI pathway resulting from a germline mutation in a mismatch repair gene and develop from traditional adenomas. The majority of MSI-H cancers, however, occur sporadically following the epigenetic silencing of *MLH1* and are thus part of the CIMP pathway, which also includes CIMP positive, MSS cancers. The CIMP pathway is usually associated with *BRAF* mutation but in *BRAF* wild-type cancers may be associated with *KRAS* mutation. These molecular features are exploited in determining which cancers are likely to have been caused by HNPCC. In the future the well-established partnership between the clinic and the laboratory will continue to identify new approaches to reduce the burden of CRC on our community.

Competing Interests: None declared.

References

1. Australian Institute of Health and Welfare (AIHW) - Australian Cancer Incidence and Mortality (ACIM) books.http://www.aihw.gov.au/cancer/data/acim_books/index.cfm (Accessed 5 January 2009).
2. Australian Cancer Network. Australian Cancer Network Colorectal Cancer Guidelines Revision Committee (ACN Revision Committee). Guidelines for the prevention, early detection and management of colorectal cancer. Sydney: The Cancer Council Australia and ACN. 2005.
3. Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, et al. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA Cancer J Clin 2008;58:130-60.
4. Issa JP. Colon cancer: it's CIN or CIMP. Clin Cancer Res 2008;14:5939-40.
5. Worthley DL, Whitehall VL, Spring KJ, Leggett BA. Colorectal carcinogenesis: road maps to cancer. World J Gastroenterol 2007;13:3784-91.
6. Rajagopalan H, Lengauer C. Aneuploidy and cancer. Nature 2004;432:338-41.
7. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001;411:366-74.
8. Loeb LA. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 1991;51:3075-9.
9. Grady WM. Genomic instability and colon cancer. Cancer Metastasis Rev 2004;23:11-27.
10. Freeman DJ, Norrie J, Sattar N, Neely RD, Cobbe SM, Ford I, et al. Pravastatin and the development of

diabetes mellitus: evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. Circulation 2001;103:357-62.
11. Takayama T, Ohi M, Hayashi T, Miyanishi K, Nobuoka A, Nakajima T, et al. Analysis of K-ras, APC, and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. Gastroenterology 2001;121:599-611.
12. Smith G, Carey FA, Beattie J, Wilkie MJ, Lightfoot TJ, Coxhead J. Mutations in APC, Kirsten-ras and p53 - alternative genetic pathways to colorectal cancer. Proc Natl Acad Sci USA 2002;99:9433-8.
13. Young GP, Rozen P, Levin B. How does colorectal cancer develop? In: Colorectal Cancer in Clinical Practice: Prevention, Early Detection, and Management. 1st Ed. London, UK: Martin Dunitz; 2002. p. 23-37.
14. Sjöblom T, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, et al. The consensus coding sequences of human breast and colorectal cancers. Science 2006;314:268-74.
15. Cadigan KM, Liu YI. Wnt signaling: complexity at the surface. J Cell Sci 2006;119:395-402.
16. Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, Yuan J, et al. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. Proc Natl Acad Sci USA 2004;101:266-71.
17. Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat Cell Biol 2001;3:433-8.
18. Jass JR, Whitehall VL, Young J, Leggett BA. Emerging concepts in colorectal neoplasia. Gastroenterology 2002;123:862-76.
19. Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. Br J Surg 2002;89:845-60.
20. Cheng L, Lai MD. Aberrant crypt foci as microscopic precursors of colorectal cancer. World J Gastroenterol 2003;9:2642-9.
21. Shen L, Toyota M, Kondo Y, Lin E, Zhang L, Guo Y, et al. Integrated genetic and epigenetic analysis identifies three different subclasses of colon cancer. Proc Natl Acad Sci USA 2007;104:18654-9.
22. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-32.
23. Bevan S, Woodford-Richens K, Rozen P, Eng C, Young J, Dunlop M, et al. Screening SMAD1, SMAD2, SMAD3, and SMAD5 for germline mutations in juvenile polyposis syndrome. Gut 1999;45:406-8.
24. Mills AA. p53: link to the past, bridge to the future [comment]. Genes Dev 2005;19:2091-9.
25. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58:5248-57.
26. Bresalier RS. Malignant neoplasms of the large intestine. In: Feldman M, Friedman LS, Sleisenger MH, editors. Sleisenger & Fordtran's Gastrointestinal and Liver Disease. 7th Ed. Philadelphia: Saunders; 2002. p. 2215-2261.
27. Weisenberger DJ, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006;38:787-93.
28. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. Gut 2004;53:1137-44.
29. Young J, Jenkins M, Parry S, Young B, Nancarrow D, English D, et al. Serrated pathway colorectal cancer in the population: genetic consideration. Gut 2007;56:1453-9.
30. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007;50:113-30.
31. Iino H, Jass JR, Simms LA, Young J, Leggett B, Ajioka Y, et al. DNA microsatellite instability in hyperplastic polyps, serrated adenomas, and mixed polyps: a mild mutator pathway for colorectal cancer? J Clin Pathol 1999;52:5-9.
32. German HNPCC Consortium, Müller A, Beckmann C, Westphal G, Bocker Edmonston T, Friedrichs N, et al. Prevalence of the mismatch-repair-deficient phenotype in colonic adenomas arising in HNPCC patients: results of a 5-year follow-up study. Int J Colorectal Dis 2006;21:632-41.
33. Hawkins NJ, Bariol C, Ward RL. The serrated neoplasia pathway. Pathology 2002;34:548-55.
34. East JE, Saunders BP, Jass JR. Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management. Gastroenterol Clin North Am 2008;37:25-46.
35. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008;135:1079-99.
36. Whitehall VL, Leggett BA. The serrated pathway of colorectal carcinogenesis. Curr Colorectal Cancer Rep 2009;5:75-83.
37. Harvey NT, Ruszkiewicz A. Serrated neoplasia of the colorectum. World J Gastroenterol 2007;13:3792-8.
38. Worthley DL, Whitehall VL, Buttenshaw RL, Irahara N, Greco SA, Ramsnes I, et al. DNA methylation within the normal colorectal mucosa is associated with pathway-specific predisposition to cancer. Oncogene 2010;29:1653-62.
39. Nakagawa H, Nuovo GJ, Zervos EE, Martin EW Jr, Salovaara R, Aaltonen LA, et al. Age-related

hypermethylation of the 5' region of MLH1 in normal colonic mucosa is associated with microsatellite-unstable colorectal cancer development. Cancer Res 2001;61:6991-5.
40. O'Brien MJ, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, et al. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. Am J Surg Pathol 2006;30:1491-501.
41. Kim YH, Kakar S, Cun L, Deng G, Kim YS. Distinct CpG island methylation profiles and BRAF mutation status in serrated and adenomatous colorectal polyps. Int J Cancer 2008;123:2587-93.
42. Park SJ, Rashid A, Lee JH, Kim SG, Hamilton SR, Wu TT. Frequent CpG island methylation in serrated adenomas of the colorectum. Am J Pathol 2003;162: 815-22.
43. Jass JR. Serrated adenoma of the colorectum and the DNA-methylator phenotype. Nat Clin Pract Oncol 2005;2:398-405.
44. Spring KJ, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, et al. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. Gastroenterology 2006;131:1400-7.
45. Mäkinen MJ. Colorectal serrated adenocarcinoma. Histopathology 2007;50:131-50.
46. Lipton L, Tomlinson I. The genetics of FAP and FAP-like syndromes. Fam Cancer 2006;5:221-6.
47. Sieber OM, Lipton L, Crabtree M, Heinimann K, Fidalgo P, Phillips RK, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. N Engl J Med 2003;348:791-9.
48. Al-Tassan N, Chmiel NH, Maynard J, Fleming N, Livingston AL, Williams GT, et al. Inherited variants of MYH associated with somatic G:C-->T:A mutations in colorectal tumors. Nat Genet 2002;30:227-32.
49. Lipton L, Tomlinson I. The multiple colorectal adenoma phenotype and MYH, a base excision repair gene. Clin Gastroenterol Hepatol 2004;2:633-8.
50. Tenesa A, Farrington SM, Dunlop MG. Re: Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. J Natl Cancer Inst 2005;97:320-1; author reply 321-2.
51. Southey MC, Jenkins MA, Mead L, Whitty J, Trivett M, Tesoriero AA, et al. Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. J Clin Oncol 2005;23:6524-32.
52. Lenz HJ. First Amsterdam, then Bethesda, now Melbourne? J Clin Oncol 2005;23:6445-9.
53. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96:261-8.
54. Lynch HT, Lynch PM, Lanspa SJ, Snyder CL, Lynch JF, Boland CR. Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. Clin Genet 2009; 76:1-18.
55. McGivern A, Wynter CV, Whitehall VL, Kambara T, Spring KJ, Walsh MD, et al. Promoter hypermethylation frequency and BRAF mutations distinguish hereditary non-polyposis colon cancer from sporadic MSI-H colon cancer. Fam Cancer 2004;3:101-7.
56. Pino MS, Mino-Kenudson M, Wildemore BM, Ganguly A, Batten J, Sperduti I, et al. Deficient DNA mismatch repair is common in Lynch syndrome-associated colorectal adenomas. J Mol Diagn 2009;11:238-47.
57. Baglietto L, Lindor NM, Dowty JG, White DM, Wagner A, Gomez Garcia EB, et al. Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst 2010;102:193-201.
58. Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 2008;135:419-28.
59. Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. J Natl Cancer Inst 2009;101:1308-24.
60. Diehl F, Li M, Dressman D, Shen D, Szabo S, Diaz LA Jr, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci USA 2005;102:16368-73.
61. Wang JY, Hsieh JS, Chang MY, Huang TJ, Chen FM, Cheng TL, et al. Molecular detection of APC, K- ras, and p53 mutations in the serum of colorectal cancer patients as circulating biomarkers. World J Surg 2004;28:721-6.
62. Zou H, Taylor WR, Harrington JJ, Hussain FT, Cao X, Loprinzi CL et al. High detection rates of colorectal neoplasia by stool DNA testing with a novel digital melt curve assay. Gastroenterology 2009;136:459-70.
63. Tagore KS, Lawson MJ, Yucaitis JA, Gage R, Orr T, Shuber AP, et al. Sensitivity and specificity of a stool DNA multitarget assay panel for the detection of advanced colorectal neoplasia. Clin Colorectal Cancer 2003;3:47-53.
64. Traverso G, Shuber A, Levin B, Johnson C, Olsson L, Schoetz DJ Jr, et al. Detection of APC mutations in fecal DNA from patients with colorectal tumors. N Engl J Med 2002;346:311-20.
65. Diehl F, Schmidt K, Durkee KH, Moore KJ, Goodman SN, Shuber AP, et al. Analysis of mutations in DNA isolated from plasma and stool of colorectal cancer patients. Gastroenterology 2008;135:489-98.