Morphology of the different molecular pathways in colorectal cancer

Jeremy R Jass
McGill University & MUHC
Overview

- Limitations of adenoma-carcinoma sequence as a genetic model for colorectal tumorigenesis
- Genetic instability & DNA methylation
- Molecular classification of CRC
  - Clinical features
  - Morphology
  - Molecular biology
Adenoma-carcinoma sequence as a genetic model of tumorigenesis

- Normal
- Small adenoma
- Large adenoma
- Cancer

Genetic instability

**Genes**
- APC
- KRAS
- TP53
### Mutation in sporadic colorectal neoplasia

<table>
<thead>
<tr>
<th>Gene</th>
<th>Dys ACF</th>
<th>TA</th>
<th>CRC</th>
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<tbody>
<tr>
<td><strong>APC</strong></td>
<td>19%</td>
<td>33%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>63%</td>
<td>11%</td>
<td>35%</td>
</tr>
<tr>
<td><strong>TP53</strong></td>
<td>0%</td>
<td>0%</td>
<td>50%</td>
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<tr>
<td><strong>B-CAT</strong></td>
<td>0%</td>
<td>13%</td>
<td>0%</td>
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Interpretation of mutational spectrum in colorectal neoplasms

- Minute adenomas initiated by \textit{APC} or \textit{KRAS}
- Mutation of \textit{APC} and \textit{KRAS} can occur during progression to CRC
- Progression to CRC is more likely when both \textit{APC} and \textit{KRAS} are mutated
- Progression more likely if \textit{APC} mutated first
- Initiation of TAs explained by multiple mechanisms, not all of which are known
- Few CRCs have mutation of \textit{APC}, \textit{KRAS} & \textit{TP53} (Smith et al, PNAS 2002;99:9433)
Molecular signatures of colorectal cancer

- DNA ploidy (aneuploid/diploid)
- Mutations (APC, KRAS, BRAF, TP53)
- Loss of heterozygosity (LOH)
- Chromosomal loss/gain (CGH)
- DNA microsatellite instability (MSI)
- DNA methylation (CIMP)
- cDNA microchip array
Demonstration of alleles by gel electrophoresis of amplified microsatellites

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>LOH</th>
<th>MSI</th>
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<tr>
<td>M</td>
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<tr>
<td>P</td>
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DNA methylation

- Cytosine in CpG sites
- CpG sites cluster as islands
- CpG island methylator phenotype (CIMP)
- CIMP: concordant methylation across multiple genes or loci
- Promoter methylation leads to gene silencing
Molecular classification of CRC

- Diploid
- Aneuploid
- MSI
- MSS
- Meth+
- Meth-
<table>
<thead>
<tr>
<th>Molecular classification of colorectal cancer</th>
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<tbody>
<tr>
<td>Meth- / MSS (1) (70%)</td>
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<tr>
<td>Meth+ / MSI (2) (15%)</td>
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<tr>
<td>Meth+ / MSS (3) (10%)</td>
</tr>
<tr>
<td>HNPCC (4) (5%)</td>
</tr>
</tbody>
</table>
Meth-/MSS (Group 1) Clinical features

- 80% in left colorectum
- Males
- Response to 5FU
CRC age adjusted incidence rates/100,000
210,452 white Americans over 21 years/SEER data

Rabeneck et al, Am J Gastroenterol 2003;98:1400
Adjuvant therapy in MSS CRC (stage II and III)

Ribic et al, NEJM 2003;349:247-257
Meth-/MSS (Group 1)
Pathology

- Few tumour infiltrating lymphocytes
- Rarely mucinous
- Dirty necrosis
- Tumor budding
- Adenoma-like cytology
- Nuclear β-catenin
β-catenin
Tumor budding
Tumor budding
Immunophenotype of tumor budding

Transformation to motile and invasive phenotype with low proliferation

Expression of: \( \beta \)-catenin, LN5-\( \gamma \)2, p16, cyclin-D1, MMP-7 (matrilysin), MT1-MMP, CD44, uPAR, P-glycoprotein, MUC1, fibronectin

Loss of: E-cadherin, reduced Ki-67 index
Meth-/MSS (Group 1)
Molecular features

- APC mutation (70%)
- KRAS mutation (30%)
- TP53 mutation (50%)
- LOH
- Aneuploidy (c.90%)
Meth+/MSI (Group 2)
Clinical features

- 90% in right colon
- Females
- Age-related
- African Americans + Whites > Asians
- Smoking
- Good prognosis
- No response to 5FU
- ?Increasing incidence in West
MSI-H status and prognosis of stage III CRC in patients who did not receive chemotherapy

Wright et al, Br J Surg 2000;87:1197-202

P = 0.015
Adjuvant therapy in MSI-H CRC (stage II and III)

Ribic et al, NEJM 2003;349:247-257
Meth+/MSI (Group 2) Pathology

- Tumor infiltrating lymphocytes
- Mucinous
- Poor differentiation
- Serration
- Expanding invasive margin
- Morphologic heterogeneity
- Little tumour budding
- Membranous $\beta$-catenin
- Serrated polyps
Poor diff/TILs
MSI-H serrated adenocarcinoma
MSI-H serrated adenocarcinoma
Mixed polyp – SSA/SSP and TSA
Serrated pathways

N → SSA

Meth & BRAF/KRAS → MLH1

SSA → MP

MGMT

MP → MSI-H CRC

MP → MSI-L/ MSS CRC
Meth+/MSI (Group 2) Molecular features

- *BRAF* mutation
- *hMLH1* methylation
- *TGFbRII, BAX, IGF2R* common
- *KRAS, TP53, APC* uncommon
- LOH uncommon
- Diploid
Meth+/MSS (Group 3) Clinical features

- 60% in right colon
- Females
- Aggressive
- Early onset
- Responsive to 5FU
- Family history
Meth+/MSS (Group 3) Pathology

- Mucinous
- Poor differentiation
- Serration
- Few lymphocytes
- Diffuse infiltration
- Serrated polyps
Serrated carcinoma in 17-year-old female
Poorly differentiated CRC with methylation of MGMT, HPP1, p16, p14, RIZ, but not MLH1
Methylation: MGMT, HPP1, p16, p14, RIZ, but not MLH1
*Meth+/MSS (Group 3) Molecular features

- *BRAF* or *KRAS* mutation
- *MGMT* methylation*
- Some are MSI-L*

*Molecular features associated with a poor prognosis (not in MSI-H CRCs)*
DNA methylation predicts chemoresponsiveness in CRC

• Stage III CRC: 103 with chemotherapy and 103 without chemotherapy (5FU)
• Relative risk = 0.22, p = 0.027
• Independent of MSI and p53 status
  van Rijnsoever et al, 2003

Majority of cases will have MGMT methylation
MGMT methylation: Good predictive/bad prognostic factor in stage II/III CRC (5 yr survival)

Chemo+/Meth+  88%*
Chemo+/Meth-  65%
Chemo-/Meth-  60%
Chemo-/Meth+  51%*

*P = 0.09

Miyakura, Jass, Gallinger
MSI-L status and prognosis of CRC

Cumulative Proportion Surviving (Kaplan-Meier)

- Complete
- Censored

- MSI-high
- MSI-low
- MSS
# Molecular classification of CRC

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
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<tbody>
<tr>
<td>Frequency</td>
<td>70%</td>
<td>15%</td>
<td>10%</td>
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<tr>
<td>Gender</td>
<td>M &gt; F</td>
<td>F &gt; M</td>
<td>F &gt; M</td>
</tr>
<tr>
<td>Proximal</td>
<td>20%</td>
<td>90%</td>
<td>60%</td>
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<tr>
<td>Ploidy</td>
<td>Aneuploid</td>
<td>Diploid</td>
<td>Diploid</td>
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<tr>
<td>MSI status</td>
<td>MSS</td>
<td>MSI</td>
<td>MSS</td>
</tr>
<tr>
<td>Methylation</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Repair def</td>
<td>?</td>
<td>MLH1</td>
<td>MGMT</td>
</tr>
<tr>
<td>BRAF</td>
<td>-</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Average</td>
<td>Good</td>
<td>Bad</td>
</tr>
<tr>
<td>Polyp</td>
<td>Adenoma</td>
<td>Serrated</td>
<td>Serrated</td>
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</table>
Lynch syndrome/HNPCC (4)
Clinical features

- 60% in right colon
- Males > Females
- Family history
- Early onset
- Multiple neoplasia
Lynch syndrome/HNPCC (4) Pathology

- Tumour infiltrating lymphocytes
- Mucinous
- Poor differentiation
- Tumor budding
- Adenomas (few)
Lynch syndrome/HNPCC
## TIL counts in H&E sections

<table>
<thead>
<tr>
<th>Sens</th>
<th>Spec</th>
</tr>
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<tbody>
<tr>
<td>3.5/5hpf</td>
<td>88%</td>
</tr>
<tr>
<td>&gt;10/5hpf</td>
<td>90%</td>
</tr>
</tbody>
</table>

* Smyrk et al, 2001
** Greenson et al, 2003
Lynch syndrome/HNPCC (4)
Molecular features

- MSI-H
- *APC* or *β-catenin* mutation
- *KRAS* mutation
- No *BRAF* mutation
- Little methylation
- Little LOH
Colorectal cancer: a set of different diseases

- Demographic features
- Risk factors
- Clinical features/behavior
- Evolutionary pathways
- Morphology
- Molecular features
- Management (diagnosis, prognosis, prevention, treatment)
Morphology of the different molecular pathways in colorectal carcinoma

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1. Introduction
After briefly reviewing to concept of colorectal cancer (CRC) as a genetic disease, some of the molecular approaches to classifying CRC will be presented, notably (a) genetic instability, and (b) DNA methylation known also as the CpG island methylator phenotype (CIMP). Genetic instability encompasses chromosomal instability (CIN) that is also described as microsatellite stable (MSS) versus microsatellite instability (MSI). On the basis of these molecular alterations it is possible to classify CRC into 4 main groups according to microsatellite status (MSS versus MSI) and CIMP status (pos/neg).

Group 1: MSS/CIMP-neg
Group 2: MSI/CIMP-pos
Group 3: MSS/CIMP-pos
Group 4: MSI-/CIMP-neg (many will be HNPCC).

The clinical, morphologic and molecular features of these four groups will be discussed.

2. Concept of CRC as a genetic disease
By studying the genetic alterations at different stages in the development of colorectal neoplasia it has been possible to construct a model of colorectal tumorigenesis. It was originally thought that all (or the ‘vast majority’) of colorectal cancers developed by the same linear sequence of genetic alterations implicating key ‘cancer genes’:

1. Inactivation (e.g. by mutation and loss) of both copies of the tumour suppressor gene \( APC \) resulting in the initiation of a small adenoma
2. Mutation of the oncogene \( K-ras \) giving a larger adenoma
3. Mutation and loss of additional tumour suppressor genes, notably \( p53 \), driving the transition from adenoma to cancer

This ‘classical’ model(1) rapidly became widely accepted dogma and encouraged the search for equivalent linear genetic pathways in other common types of malignancy. To date, this search has been unsuccessful. Moreover, critical examination of the evidence underlying the ‘classical’ model for CRC has exposed a number of problems.

Problems with genetic model for the adenoma-carcinoma sequence

\( APC \) gene: It was originally assumed that the autosomal dominant disorder familial adenomatosus polyposis (\( FAP \)) represented the genetic counterpart or all (or the ‘vast majority’) of sporadic CRCs. Patients with \( FAP \) carry a germline mutation of \( APC \) in every cell and all adenomas (even of microscopic size) obtained from patients with \( FAP \) will have bi-allelic inactivation of \( APC \) with either mutation or loss of the second copy. It was therefore assumed that sporadic colorectal adenomas occurring in the general population must also have bi-allelic inactivation of \( APC \) (even if this could not be
demonstrated in practice). If this were true then 100% of CRCs should carry an APC mutation. In fact the actual figure is closer to 60% (higher for left-sided CRCs and lower for right-sided CRCs).(2)

\textbf{\(\beta\)-catenin:} Inactivation of APC results in the stabilization of the Wnt pathway transcriptional activator \(\beta\)-catenin which then translocates to the nucleus and in turn leads to the expression of multiple oncogenes (e.g. c-myc) and additional genes implicated in tumour invasiveness. Point mutation of \(\beta\)-catenin may lead to its oncogenic activation that would have the same effect as APC mutation and could therefore substitute for APC mutation. In fact, only a subset of CRCs occurring in the rare autosomal dominant condition HNPCC have mutation of \(\beta\)-catenin.(3)

\textbf{K-ras:} Around 60% of large adenomas have mutation of K-ras. Yet only around 30% of CRCs have mutation of this oncogene leading to activation of the MAP-kinase signaling pathway.(2)

\textbf{p53:} While it is true that around 70% of CRCs show chromosomal losses in 17p close to the locus for p53, mutation of p53 occurs in only around 40-50% of CRCs.(2)

\textbf{APC, K-ras and p53:} How many CRCs in fact carry mutations in the three key cancer genes APC, K-ras and p53? A recent study found all three alterations in only 7% of CRCs.(4) While the concept of a dominant ‘classical’ molecular pathway to CRC explained by alterations of the three genes APC, K-ras and p53 is widely believed and widely published, it is not backed by strong evidence.

3. Defining tumorigenic pathways on the basis of genetic instability
It is likely that the acquisition of the state of \textit{genetic instability} is an important step in tumorigenesis, particularly in cancers that evolve through the accumulation of multiple genetic alterations. Normal cells do not tolerate DNA or chromosomal damage. They either repair the damage or signal self-destruction by apoptosis. Genetic instability is a state that requires two conditions:

(1) An increase in the rate of DNA or chromosomal damage (mainly occurring during cell division – a vulnerable time for a cell), and
(2) Failure to initiate apoptosis in the face of mounting DNA or chromosomal damage.

Damage does not necessarily mean mutation - most mutations are in fact silent (though some mutations result in genetic damage - see below). DNA damage refers, for example, to:

(1) Chemical alterations to DNA by addition of chemical adducts,
(2) Mismatches between template DNA and the replicated copy, or
(3) Major alterations in the number of chromosomes.
The cell has mechanisms for detecting and repairing these types of damage or for initiating apoptosis if the damage is beyond repair. Cancer cells that continue to proliferate despite accumulating genetic damage of this sort are characterized by the state of genetic instability. The reason why genetic instability is so important in tumorigenesis is because spontaneous mutations that confer a biological growth advantage occur very infrequently. An increased rate of DNA damage will increase the mutation rate, but is a two-edged sword. While DNA damage may result in a mutation that confers a growth advantage it is much more likely that it will trigger apoptosis because the cell is unable to repair the genetic damage. Very few cells will acquire the full set of mutations that result in malignant transformation.

Some DNA repair genes (for example some DNA mismatch repair genes) not only repair DNA but also initiate apoptosis when they cannot repair the damage. Therefore, the inactivation of a DNA repair gene is a very potent method of initiating a cancer. Not only will the cell acquire large amounts of DNA damage but it will also have lost the mechanism for detecting the damage and triggering apoptosis. By a single stroke there will be an increased rate of DNA damage and also inhibition of apoptosis. This is the perfect combination for the efficient initiation of cancer.

4. Chromosomal instability (CIN)

It has been known since the 1980s that approximately 70% of CRCs are aneuploid or comprise abnormal numbers of chromosomes (approximately 50% more chromosomal material than is found in normal cells). Conversely, 30% of CRCs are diploid (or near-diploid) with a chromosomal content similar to normal cells. Methods for demonstrating such chromosomal instability (CIN) include DNA flow cytometry, direct karyotyping and comparative genomic hybridization.

CRCs with CIN show frequent loss of tumor suppressor genes. This may be demonstrated by a variety of DNA recombinant technologies but most simply by using the polymerase chain reaction (PCR) to replicate DNA segments that include microsatellite sequences. Microsatellites are repetitive non-coding DNA sequences that may be mononucleotide (e.g. AAAAAAAA), dinucleotide (e.g. CACACACA), or trinucleotide (e.g. CAGCAGCAG). The lengths of these repetitive sequences vary from one person to another, i.e. they are polymorphic and can therefore be used for DNA ‘fingerprinting’ and linkage analysis within families. Polymorphic microsatellite markers may be able distinguish maternal and paternal DNA sequences from an individual subject, thereby establishing that the individual is heterozygous for that particular DNA sequence. In DNA derived from a tumor developing in such a heterozygote subject it may be possible to show that there has been loss of either the maternal or paternal sequence. This is described as loss of heterozygosity (LOH). If a particular type of tumour shows consistent LOH at a specific genetic locus then it can be inferred that there is a tumour suppressor gene, for example APC or p53, close to that locus.

5. Microsatellite instability (MSI)

In the process of testing for LOH in CRCs during the 1990s it was noticed that some tumours did not have LOH. Instead, the microsatellite markers showed band-shifts
indicative of alterations in the lengths of microsatellite markers due to insertion or deletion type mutations. These band-shifts were markers for a subtle type of genetic instability at the DNA level. This has been called microsatellite instability (MSI) because it occurs in microsatellite sequences and is demonstrated with the aid of microsatellite markers.\(^{(5, 6)}\) The repetitive tracts of microsatellite DNA are hotspots for DNA mismatches. The latter are normally detected and repaired by DNA mismatch repair (MMR) proteins encoded by the genes MLH1, MSH2, MSH6 and PMS2. If the genes encoding DNA MMR proteins are inactivated then mismatches will not be repaired, but will generate insertion or deletion type mutations that are the hallmark of MSI.

It is usual to employ five microsatellite markers to detect MSI. The demonstration of bandshifts in at least two of the five markers is defined as high-level MSI (MSI-H) and implies that there is inactivation of a DNA MMR gene. Bandshifts involving only one of five markers denote low-level MSI (MSI-L). MSI-L does not indicate a defect of DNA mismatch repair per se but has been linked with the generation of excessive numbers of mismatches through loss of function of the DNA repair gene MGMT. Immunohistochemistry using monoclonal antibodies to the DNA MMR proteins provides an alternative method for detecting MMR deficiency, i.e. by showing loss of a particular MMR protein within the cell nucleus.

The inactivation of DNA mismatch repair genes may come about in two principal clinical situations:

1. Germline mutation giving the autosomal dominant condition known as Lynch syndrome or Hereditary Non-Polyposis Colorectal Cancer (HNPCC) that accounts for around 2% of all CRC.
2. Acquired inactivation at the somatic level through methylation of the promoter region of MLH1 that accounts for around 15% of sporadic CRC.

6. DNA methylation in colorectal cancer

Just as a panel of DNA microsatellite markers can be used to identify MSI-H and MSI-L status in CRCs, so can a panel of genes and other anonymous loci be used to demonstrate DNA hypermethylation. Around 50% of genes have clusters of the dinucleotide cytosine-guanine (CpG) within their promoter regions that are termed CpG islands. Elsewhere in the genome cytosine within CpG sites is hypermethylated but in the promoter region the CpG islands are normally non-methylated. When they become methylated the gene can no longer be transcribed. This provides a novel epigenetic mechanism of gene silencing (implying that gene inactivation has occurred without structural changes to gene, i.e. mutation). The list of potential tumour suppressor genes that can be inactivated by hypermethylation is given below (see Group 2 CRC).\(^{(7)}\)

CRCs with extensive DNA methylation are said to have the CpG island methylator phenotype (CIMP).\(^{(8)}\) CIMP-Positive CRCs include the sporadic MSI-H subset (accounting for up to 15% of CRC) and termed Group 2 CRC (see below). Up to 15% of CIMP-Pos CRCs are not MSI-H and are termed Group 3 (see below). The underlying mechanisms of DNA methylation are still poorly understood but there is evidence for a
genetic (hereditary) predisposition to DNA methylation. Interestingly, the precursor lesion for CIMP-positive CRC is likely to be a lesion that has been regarded as harmless for many decades: the hyperplastic polyp. Attention is now being focused on large, right-sided hyperplastic polyps. Many of these polyps are CIMP-positive and have mutation of the oncogene BRAF. These lesions may differ histologically in subtle ways from the common, small and largely harmless hyperplastic polyp found in the distal colon and rectum and several new terms for them has been introduced, e.g.: ‘sessile serrated adenoma’, ‘sessile serrated polyp’, and ‘serrated polyp with atypical proliferation’.

7. Molecular classification of colorectal cancer
The 2005 edition of Robbins describes two distinct types of CRC based on molecular pathology and other features. The following summarizes the main features of these two groups but highlights a third group and also a fourth group represented by HNPCC.

Group 1 CRC accounts for approximately 70% of CRCs. This group shows CIN as evidenced by DNA aneuploidy and frequent loss of tumour suppressor genes. These Group 1 CRCs have a number of clinical and pathological features that distinguish them from the other types of CRC:

- More common in males
- Most occur in left colon or rectum
- Mainly well or moderately differentiated adenocarcinoma
- Frequent ‘dirty necrosis’
- Few tumour infiltrating lymphocytes
- Tumour budding (de-differentiation) at invasive front of tumour
- Origin within adenoma
- Little DNA methylation (see below)

Group 2 CRCs are diploid, MSI-H and CIMP-Positive and are characterized by the following clinical, pathological and molecular features:

- Late age at onset*
- More common in females*
- More likely to occur in proximal (right) colon
- More likely to be poorly differentiated
- More likely to secrete mucin
- Circumscribed invasive margin with little tumour budding
- Associated with tumour infiltrating lymphocytes (CD8+)
- Associated with serrated polyps (hyperplastic polyps, mixed polyps and serrated adenomas)*
- Express both intestinal (MUC2) and gastric (MUC5AC and MUC6) core mucin proteins
- Mutations of APC, K-ras and p53 are absent or very uncommon*
- Mutation of: BRAF*, TGFβRII, BAX and IGF2R are common
Frequent methylation of the promoter regions of the following genes: MLH1, MGMT, HPP1, RASSF1, RASSF2, RUNX3, p16INK4a, p14ARF, CACNA1G, SLC5A8, TIMP3, THBS1, FHIT, HIC1, DAPK, FASR, etc*

- Has good prognosis
- Shows no response to chemotherapy (use of chemotherapy may even be associated with increased mortality)

*The asterisked features are not found in HNPCC (see below) indicating that sporadic MSI-H CRC and HNPCC are not exact equivalents but evolve through independent pathways.

**Group 3** CRCs are diploid, CIMP-Positive but not MSI-H and show features intermediate between **Group 1** and **Group 2** CRC(10-15):

- More common in females than males
- Predilection for proximal colon
- May show early age at onset and positive family history
- Often mucinous and/or poorly differentiated
- Few tumour infiltrating lymphocytes
- **MSI-L** is frequent
  - Association with serrated polyps (hyperplastic polyps, mixed polyps and serrated adenomas)
  - Mutation of K-ras or BRAF
  - Frequent methylation of *MGMT*, p14ARF and other loci (particularly when right-sided)
  - Frequent LOH and mutation of APC and p53
  - Poor prognosis (particularly when right-sided)
  - Possible good response to chemotherapy (particularly with MGMT methylation)

*O-6 Methylguanine DNA Methyltransferase (MGMT) is a DNA repair gene that removes damaging methyl adducts from guanine. Loss of function of MGMT results in methylG:T mismatches and mutations in K-ras and p53 and may account for MSI-L status and a form of CIN associated with LOH but not aneuploidy.(16)

CRCs in **HNPCC** or **Lynch syndrome** are MSI-H but differ from **Group 2** CRCs as outlined above and with respect to the following features:

- More common in males
- Predilection for proximal colon but not to same extent as sporadic MSI-H CRC
- Early onset (mean around 45 years)
- Multiple CRC
- Strong family history
- Extra-colonic malignancy (endometrium, ovary, pelvi-ureter, stomach, CNS)
- Origin within adenoma
- Mutation of APC or β-catenin
- Little DNA methylation
- No mutation of BRAF

8. Conclusion

While there is considerable interest in developing molecular classifications based on cluster analysis of very large amounts of molecular data obtained through various bio-informatic approaches, it is clear that clinical meaningful groupings can be achieved on the basis of relatively simple matrices involving two molecular features: genetic instability and DNA methylation. The four CRC groups identified by this approach differ with respect to demographics, clinical features, prognosis, response to therapy, molecular profiles and multiple morphologic features that can be readily recognized by pathologists. This suggests that genetic instability and DNA methylation are early and fundamental alterations that initiate independent tumorigenic pathways. Furthermore, the outlined approach is very much in the tradition of classic clinical-pathological-molecular correlation and is inherently more satisfying for pathologists than attempting to extract a classification from a bio-informatic back-hole.

References


