

Update of Genetics in Colorectal Carcinomas: Genomic Instability and Somatic Evolution

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Abstract

Introduction: Currently, there are two contrasting viewpoints on what drives the process of carcinogenesis. The genomic (DNA or chromosomal) instability model contends that an increased mutation rate early in carcinogenesis is necessary for the multistage process, while the somatic evolution model postulates that normal mutation rate with selective advantage and clonal expansion is sufficient to cause cancer. **Methods:** Evidence from colorectal carcinoma (CRC) for and against the two models are compared and contrasted. **Results:** With the exception of hereditary non-polyposis colorectal carcinoma (HNPCC) where DNA instability attributable to mismatch repair deficiency is clearly demonstrated, the majority of CRC appear to progress through the selection of a series of mutations without the need of first acquiring a mutator phenotype. Aneuploidy or chromosomal instability is more likely to be a consequence of non-random selection of mutations in genes residing on the chromosome rather than the direct cause of cancer. Nevertheless, aneuploidy and / or DNA alterations can lead to secondary instability, hence, contributing to the phenotypes associated with carcinoma. **Conclusions:** Present knowledge, thus, points to multiple, mutually non-exclusive pathways for different cancer populations, further emphasising tumour heterogeneity.

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Introduction

The hypothesis that carcinogenesis occurs through stepwise accumulation of mutations¹ has now been generally accepted. However, there is continual dispute over how carcinogenesis can be achieved over the lifetime of an individual. Proponents of the genomic instability model contend that spontaneous mutation rate in somatic cells is not sufficient to account for the multiple mutations observed in tumours, and that an early step in tumour progression is the acquisition of an increased mutation rate or the 'mutator phenotype'.²⁻⁴ Conversely, carcinogenesis is viewed as a process of somatic evolution.^{5,6} Tumour growth is initiated by mutation that confers selective advantage to the cell which then expands clonally. Successive advantageous mutations are selected, each followed by waves of clonal expansion. This is shown to be both necessary and sufficient for carcinogenesis and there is no necessity for an increased mutation rate.⁵

We review here evidence from colorectal carcinoma (CRC) for and against the two models and conclude that the two apparently disparate views can be reconciled in a

manner that provides insight into mechanisms underlying the carcinogenesis process.

Genomic Instability Model

The proponents of this model argued that most cancers are inherently unstable and that the instability exists at two distinct levels.^{4,7} In a small subset of tumours, the instability occurs at the DNA or nucleotide level. For the majority of tumours, the instability is observed at the chromosomal level, resulting in aneuploidy.

The best evidence for DNA instability occurring early in tumourigenesis comes no doubt from the dominantly inherited hereditary non-polyposis colorectal carcinoma (HNPCC) which constitutes about 5% to 10 % of all CRC. HNPCC patients have few polyps in their colon but nevertheless have an accelerated rate of CRC.⁸ They also have increased rates of endometrial, urinary and ovarian tumours. In 1993, it was discovered that HNPCC patients exhibit replication error (RER) or microsatellite instability (MI) phenotype^{9,10} which is characteristic of mutation in a class of DNA repair genes called mismatched repair (MMR).

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When the MMR genes are mutated, there is no repair of replication slippage which occurs frequently at regions of mono-, di- or trinucleotide repeats and this causes replication error and hence increased mutation rate. Since then, at least six human MMR genes have been cloned by virtue of their homology to the bacteria and yeast genes.^{11–15} The *hMSH2* and *hMLH1* genes account for the majority of the HNPCC defects (65%) while the *hPMSH1* and *hPMSH2* genes account for another 10%. RER in HNPCC tumours is shown to be an early phenomenon, commonly occurring at the adenoma stage.^{10,16,17}

Increasing evidence suggest that carcinogenesis in HNPCC patients progress along a pathway different from that initiated by the inactivation of the adenomatous polyposis coli (*APC*) tumour suppressor gene (Fig. 1). One study¹⁶ has indicated that HNPCC tumours have significantly less mutations in *APC*, *p53* and *k-RAS* but significantly more transforming growth factor β -receptor type II (*TGF β RII*) mutations than non-HNPCC tumours. Another study¹⁸ has shown that concomitant occurrence of *APC* and *TGF β RII* mutations in RER tumours is rare. Nevertheless, one study¹⁹ showed that about equal number (50%) of RER and non-RER CRC tumours have mutations in the *APC* gene, though RER tumours have significantly more frameshift mutations which are indicative of cells deficient in DNA mismatch repair. The authors concluded that the RER phenotype precedes, and is responsible for the *APC* mutation in RER tumours. They, however, have not made a distinction between HNPCC and non-HNPCC RER tumours. Therefore, it is as yet unclear whether the majority of the HNPCC tumours initiate with mutation in the *APC* gene. The literature is also confounded by the varying definitions of RER by different authors.^{20–22} Interestingly, a recent study²³ has found a high frequency of β -catenin mutation in HNPCC tumours without *APC* mutation indicating that mutation in the former may substitute for the latter in the initiation of HNPCC tumourigenesis.

The genomic instability model predicts that the majority

of the CRC without DNA instability have chromosomal instability or aneuploidy.⁴ Indeed, a large proportion of, though not all, non-RER tumours are aneuploid (see section below). Recently, Rasnick and Duesberg²⁴ put forward an argument that complex and diverse cancer phenotypes are due to aneuploidy over a certain threshold. The authors argued that there is as yet no functional proof that any one or a combination of the 7 genetic mutations said to cause CRC can generate cancer specific phenotypes such as de-differentiation, invasiveness, metastasis, abnormal morphology and metabolism and genetic instability compared with normal cells. Aneuploidy, in contrast, results in massive changes in dosage of gene products that can alter the physiology and metabolism of cells resulting in these phenotypes. They further argued in their mathematical model that it is the fraction of the genome undergoing differential expression (that is, extent of aneuploidy) and not the magnitude of the differential expression that controls the phenotypic transformation.

Though the aneuploid model is attractive, it nevertheless does not provide convincing evidence on how aneuploidy occurs and more importantly how it is maintained or accumulated in the cell to a threshold to cause cancer. A whole range of genetic defects can lead to aneuploidy including those involved in centrosome replication,^{25–28} mitotic ‘checkpoints’^{29–31} and sister chromatid cohesion and cleavage.^{32,33} The fact that so many diverse genetic defects can lead to chromosomal instability suggests a heterogeneous basis for aneuploidy.⁷ This lends support to the somatic evolution model that contends that aneuploidy is a ‘bystander’ or ‘hitch-hiker’ to some other functions for which the diverse genes have been selected (see section below). Besides, aneuploidy alters gene dosage, not gene function, while ‘function-changing’ mutations are necessary for carcinogenesis.³⁴ It is also inconceivable that a cell with a heavy aneuploidy load does not die by apoptosis if there is no selective advantage to maintain it. The model also implies that aneuploidy occurs in a random fashion.³⁵ There are evidence to show that chromosomal gains and losses in

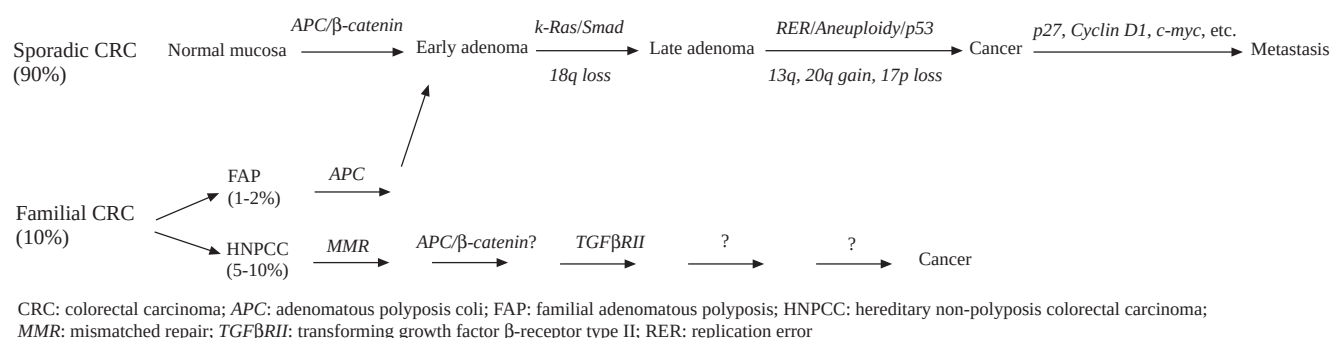


Fig. 1. A modified multistage colorectal carcinogenesis model. Two distinct genetic pathways can be delineated. One pathway is for sporadic CRC and FAP; the other is for HNPCC. The genes/chromosomes listed are by no means exhaustive and there are many missing links denoted by question marks.

CRC are not random. A recent study utilising comparative genomic hybridisation analysis³⁶ has shown that progression from adenoma to carcinoma is associated with non-random gain of chromosome 13q, 20q and 8q, thus suggesting the relative importance of oncogenes residing on these chromosomal arms.

Somatic Evolution Model

This model contends that carcinogenesis is an evolutionary process at the somatic level, in which the population is composed of cells within the tumour. Mutations occur at random and natural selection acts to increase the frequency of advantageous mutations in the population. Given that the rate of cell turnover (the whole intestinal epithelium replaces itself approximately every 3 to 4 days) far exceeds the net rate of tumour growth (which on average takes 20 or more years in sporadic tumours), and the existence of clonal expansion, there is no necessity to evoke a raised mutation rate to explain tumourigenesis:⁶ selection is both necessary and sufficient. The power of selection is eloquently demonstrated in the observation³⁴ that the A10 tract of the *TGF β RII* in RER CRC, but not RER endometrial cancer, is frequently mutated, showing that despite being hypermutable, selection pressure probably keeps the gene intact in the latter. Besides, a raised mutation rate or genomic instability does not in itself cause cancer. It can accelerate the carcinogenesis process (as has been shown in the HNPCC tumours), but it is not necessary for it to occur.

RER tumours, which reflect an underlying DNA or nucleotide instability, constitute about 10% of all sporadic CRC.²² Several studies, however, have shown that RER is rare in adenomas, even in those that are larger than 1 cm.^{10,16,37,38} This leads to the suggestion that, in contrast to HNPCC tumours, RER in sporadic colonic neoplasia occurs at the late adenoma or early carcinoma stage. In one study,¹⁶ 95% of HNPCC tumours at all stage showed RER while RER in FAP and sporadic tumours increased from 3% in adenoma to 24% in invasive carcinoma to 35% in liver metastasis, indicating that RER occurs from an early stage in HNPCC but in a later stage in non-HNPCC tumours. A recent study³⁹ also showed no significant increase of frameshift or short repeats mutations, which are characteristics of an underlying MMR deficiency, in the *APC* locus for the sporadic RER tumours compared to the non-RER tumours. This suggests that, contrary to HNPCC tumours, RER occurs after *APC* mutation for the sporadic tumours. In addition, a number of studies have concurred that the majority of the RER sporadic tumours do not have mutation in the MMR genes.^{16,22,40–42} In one study,¹⁶ only 6% of RER non-HNPCC tumours had mutation in either *hMSH2* or *hMLH1* gene. A later study²² found no evidence of mutation in *MSH2*, *MLH1*, *MSH3* or *MSH6* genes in the

sporadic RER tumour specimens (which the authors defined as 'mild RER'). Taken together, current evidence indicate that RER in sporadic CRC is not only occurring later than in HNPCC tumours, but is also likely to involve other genes and tumourigenic pathway.

Though aneuploidy is common in CRC, several studies^{43,44} have found that a substantial proportion of the tumours (about 20%) are both non-RER and non-aneuploid i.e. diploid, suggesting that neither form of genomic instability can be evoked to explain the formation of these tumours. Furthermore, while a balanced gain or loss of chromosome has been shown³⁶ in diploid tumours (including all adenomas studied), evidence has shown that gross changes in chromosome numbers (i.e. aneuploidy) occur late in the adenoma-carcinoma sequence. Aneuploidy is shown to be significantly more frequent in dysplastic adenomas than non-dysplastic adenomas.⁴⁵ This result is confirmed in a more recent study that shows a highly significant positive correlation between aneuploidy and adenoma size, architecture, and grade of dysplasia.⁴⁶

Thus, DNA or chromosomal instability, if it occurs, is probably relatively late in most sporadic CRC and contributes only to secondary instability that helps to explain the various cancer phenotypes alluded to earlier.²⁴ While the somatic evolution model contends that selection alone is sufficient for carcinogenesis to occur, in reality, the majority of the CRC probably acquire an increased mutation rate (e.g. through mutation in *p53*, RER or aneuploidy) as carcinogenesis progresses. Using the technique of inter-(simple sequence repeat) PCR, one recent study⁴⁷ had shown that genomic destabilisation was considerably more prevalent than expected in sporadic CRC. The mean genomic alterations per carcinoma cell was estimated to be approximately 11,000 and the degree of instability in a limited number of polyps (albeit of unknown sizes) was only slightly less than that in the corresponding tumours. These results support the contention that genomic instability has a role to play in accelerating somatic cell evolution. With the exception of HNPCC, however, genomic instability is unlikely to be present when tumour initiates and therefore not necessary for tumour initiation.

A Modified Multistage Model for Colorectal Carcinogenesis

Based on the evidence reviewed, we present a modified multistage model of colorectal carcinogenesis (Fig. 1). However, it should be stressed that this is just a framework to aid us in understanding a very complicated process of which there are still many missing links and should be treated as such. For simplicity, the role of modifier genes and the environment, notably diet,⁴⁸ which may affect carcinogenesis through mechanisms such as selection of

apoptosis resistant clones,⁴⁹ will not be discussed. Similarly, the role of methylation or epigenetic changes in carcinogenesis, which is another controversial issue, will not be addressed here.⁵⁰

The majority of the sporadic tumours are initiated by mutation in the *APC* locus.⁸ Alternatively, β -catenin, the target for *APC* inactivation in the T-cell transcription factor (Tcf) signalling pathway, has been shown to be able to substitute for *APC* mutation in half of the sporadic CRC tumours lacking detectable *APC* mutation.⁵¹ Two hits of either gene are required for tumour initiation. Activation of the *k-Ras* oncogene followed by the loss of heterozygosity in chromosome 18q or mutations in the *Smad* genes³⁴ allow tumour progression to the late adenoma stage. At this stage, the non-random selection of other oncogenes (yet to be determined), which causes chromosomal segregation or mitotic spindle defects in addition to conferring growth advantage, can lead to the initiation of aneuploidy. In the case of RER sporadic tumours, mutation in genes other than the MMR (such as *DNA polymerase*)⁵² could occur here. Mutation in the *p53* gene could also lead to secondary genomic instability.⁵³ It is this instability that transforms adenoma to carcinoma and contributes to the various cancer phenotypes (Fig. 1). These different mechanisms for generating instability are not necessarily mutually exclusive and could be complementary. The transformation of localised carcinoma to metastasis requires the activation or mutation of a whole series of other genes.⁵⁴ Genes involved in cell-cycle, such as *c-myc*,⁵⁵⁻⁵⁷ *p27*⁵⁸⁻⁶¹ or *cyclin D1*,⁶²⁻⁶⁴ have been shown to be deregulated in CRC, contributing to tumour progression and metastasis. The involvement of NM23 in metastasis suppression, however, is questioned in a recent study,⁶⁵ as NM23 expression was not significantly correlated to the 5-year overall survival of CRC patients.

The initiation of carcinogenesis in familial adenomatous polyposis (FAP) patients through the inheritance of a germline mutation and an acquired somatic mutation in *APC* is well documented.^{8,66-68} However, no β -catenin mutation was found in FAP patients without detectable *APC* germline mutation.^{69,70} It is therefore likely that germline mutation in β -catenin is lethal and cannot substitute for *APC* mutation in the initiation of tumourigenesis for FAP patients. Interestingly, a recent study⁷¹ found that allelic loss in *APC* is a more common 'second hit' than truncating mutations in FAP patients with germline mutations between codons 1250 and 1450. This implies that spontaneous changes that lead to allele loss could occur more frequently, thus raising the somatic mutation rate in these patients and contributing to disease severity. Hence, for FAP, an increased mutation rate (albeit by a yet unknown mechanism), dependant on the selective

advantage of the first hit in *APC*, could have occurred as early as the second hit in *APC*. Other than the mechanism of initiation which explains the difference in the number of adenomas in FAP and sporadic CRC, carcinogenesis in FAP is thought to follow essentially the same pathway as the sporadic CRC⁸ (Fig. 1).

For HNPCC patients with germline mutation in MMR, the probability of getting another hit in the same MMR gene is higher than getting 2 hits in a tumour suppressor gene. Thus, the intrinsic mutation rate in these patients is probably raised before tumour initiation. Nevertheless, 2 hits in a tumour suppressor gene (*APC*, β -catenin or other candidate gene—see section on genomic instability) are still necessary for tumour initiation as MMR gene increases the general mutation rate of the cell, but cannot initiate tumourigenesis by itself.⁵ HNPCC patients are also known to have higher frequency of mutations in genes other than those normally mutated in the sporadic pathway such as *TGF β R2* and conversely, lower mutation rate in genes such as *k-Ras* and *p53*.¹⁶ It is likely that hypermutability results in more frequent mutations in genes with simple repeats tracts (such as *TGF β R2*) than corresponding genes in the same pathway (such as the *Smad* genes) for the sporadic CRC.³⁴ It has been shown that *TGF β R2* gene alterations are early events in HNPCC tumourigenesis¹⁷ but a relatively late event in sporadic CRC and even then, it is restricted to those with extensive RER.³⁷ HNPCC tumours have also been shown to be generally diploid and have better prognosis.^{43,72,73} Thus, HNPCC tumours probably progress along a pathway distinct from the sporadic CRC (Fig. 1). Some studies have suggested that the acquisition of MMR deficiency could have precluded other mechanisms of instability such as aneuploidy and *p53* mutation.⁴ It is not yet clear whether the mechanisms of metastasis are also precluded, hence, resulting in better prognosis of these tumours.

Not much is really known about the genetic pathways of other related syndromes, such as the ulcerative colitis associated colorectal cancer,⁷⁴ hereditary mixed polyposis syndrome⁷⁵ and mixed hyperplastic adenomatous polyposis,⁷⁶ which together account for less than 5% of the total incidence of all cases of CRC, and hence, they will not be reviewed here.

Conclusion

Despite our desire to reduce the carcinogenesis process into simple linear models for ease of comprehension, current evidence point to the existence of complex, mutually non-exclusive pathways for different cancer populations. This serves to remind us again that carcinogenesis is essentially a very heterogeneous process and that it is important not to take a simplistic approach. Although

tremendous progress has been made since the multistage model of carcinogenesis was first proposed a decade ago, much effort is still needed to fill the gaps in our quest for the whole picture.

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