

Colon Cancer: It's CIN or CIMP

□□ Commentary on Cheng et al., p. 6005

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Combined genetic and epigenetic analysis of sporadic colon cancer suggest that it can no longer be viewed as a single disease. There are at least three different subsets with distinct clinicopathologic features, with important implications for preventions, screening, and therapy.

Genetic instability is an important engine of molecular diversity in neoplasia which is a prerequisite for Darwinian selection that characterizes tumor formation and progression. Nowhere is it clearer than in colon cancer (1), where landmark experiments suggested that virtually all cases had genetic instability, either of microsatellite sequences through loss of mismatch repair (these cases are often called MSI for microsatellite instability) or of large sections of chromosomes through as yet undefined mechanisms (a phenomenon termed CIN for chromosomal instability). This simple model suggests two paths to colon cancer, with all cases having some measurable degree of genetic instability.

The picture became more complex when it was reported that some colon cancers seemed to have neither MSI nor CIN (2). In parallel, epigenetic changes marked by DNA methylation were increasingly recognized as very common in cancer, and a specific pathway of intense DNA hypermethylation was described in colon cancers, the CpG island methylator phenotype (CIMP; ref. 3). Given the functional equivalence of epigenetic silencing and inactivating mutations (4), epigenetic instability provided a theoretical alternative to genetic instability in driving the molecular evolution of cancer. The issue was further complicated by the finding that most cases of sporadic MSI colon cancer could be attributable to CIMP-related silencing of the mismatch repair genes *MLH1* (3).

In this issue of *Clinical Cancer Research*, Cheng et al. (5) report on a careful analysis of the molecular profiles of 161 colon cancers; they find an inverse relationship between CIN and CIMP, suggesting that the two distinct mechanisms of generating molecular diversity rarely overlap. The data constitute an independent confirmation of a prior study that used different molecular techniques for characterization of the cancers (6). This inverse correlation makes biological sense in that selective pressures really require only one way to get to the goal, and furthermore, provides evidence for the equivalence of

genetic and epigenetic mechanisms in altering pathways in cancer. More broadly, the data have to be viewed in the context of increasing evidence for fundamental clinicopathologic differences between CIMP-positive and CIMP-negative cases (7). As (now) compared with CIN cases, CIMP cases tend to occur in the proximal colon and in older patients (with a slight female bias). They have a distinct histology that reflects distinct precursor lesions. Importantly, they have very different genetic characteristics. In addition to the differences in genetic instability, CIMP cases tend to have *BRAF* and *KRAS* mutations and fewer *APC* and *p53* mutations. Of course, one can occasionally see the coexistence of pathways in the same tumors. Cheng et al. found some cases with extensive DNA methylation and CIN, as well as cases with both MSI and CIN. It remains to be seen whether these represent yet other subgroups, or are related to disease progression (or to technical failings). One would predict that these cases, although rare, may have particularly poor outcomes.

The new data presented by Cheng et al. (5) provide further evidence for a revision of the classic model of colon cancer evolution (the "Vogelgram"). Rather than a single pathway initiated by biallelic *APC* loss, and characterized by sequential accumulation of mutations, colon cancer seems to be best characterized by at least three parallel Vogelgrams, each of which has distinct clinicopathologic features (Fig. 1). Although the revised models are a better fit for the existing data on molecular profiling of colon cancer, there are gaping holes in our knowledge, from the mechanisms underlying CIMP and CIN, to the molecular events critical for metastasis. One of the intriguing questions raised by the data is whether the three pathways initiate in identical cells, or whether there are truly three different colon cancers, in the same way that leukemias, once lumped into one big group of diseases, are now exhaustively classified based on initiating cell characteristics and/or differentiation status.

The clinical implications of multiple pathways to colon cancer should be obvious, from prevention to screening to therapy. From a prevention standpoint, if the different colon cancers arise from different cells, it is likely that familial predisposition and carcinogenic exposures affect each pathway differently. This heterogeneity in the disease may explain the difficulties in reproducing some associations between exposures, lifestyle, and genetic polymorphisms on colon cancer risk. Indeed, geographic variation in the type of colon cancer may explain the differences seen in the disease in different countries (8). By extension then, interventions to prevent colon cancer may have a preferential effect on one disease type but not on the others, an effect that might lead to false-negative

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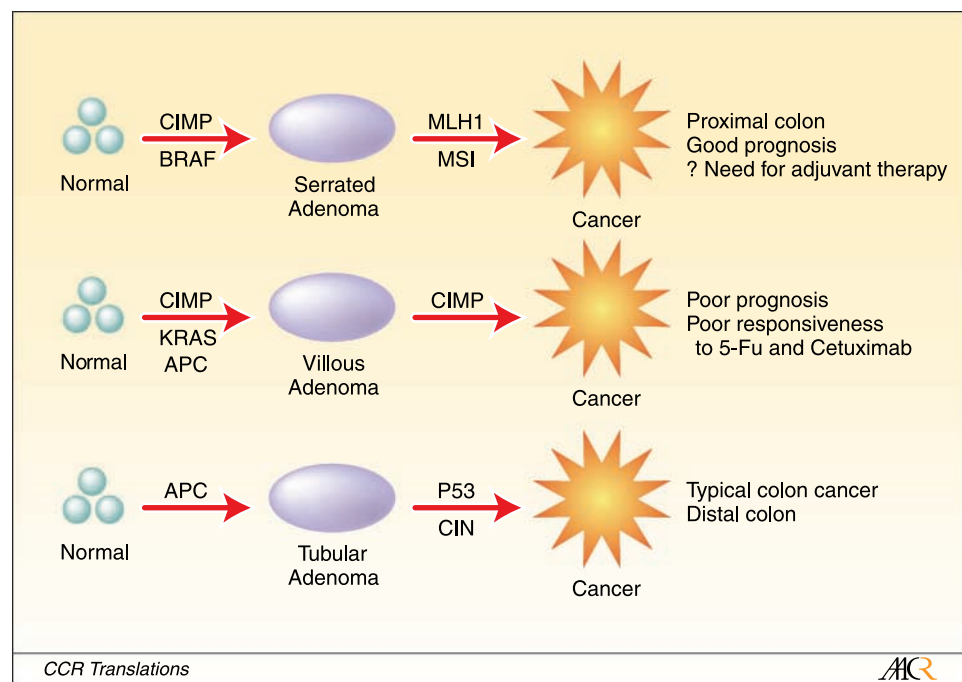


Fig. 1. Multiple parallel pathways to colorectal cancer, with clinical implications for therapy. Instead of a linear, single progression model, sporadic colorectal cancer seems to arise from (at least) three distinct parallel modes. The top and bottom pathways are the most homogeneous, with clear distinctions in precursor lesions (serrated vs. tubular adenomas), genetics (BRAF vs. APC and p53 mutations, MSI vs. CIN), epigenetics (CIMP positive vs. negative) and outcome (good vs. average). The middle pathway is more heterogeneous than depicted (or perhaps incompletely understood). It may arise mostly from villous adenomas, but perhaps also from serrated adenomas. It has a different form of CIMP, predominant KRAS but occasional BRAF mutations, usually lacks CIN, and has the worse prognosis, with apparently lower responsiveness to chemotherapy. The prevalence of the three pathways is estimated at 10% to 20% (*top pathway*), 10% to 30% (*middle pathway*), and 50% to 70% (*bottom pathway*).

trials (by dilution). From a screening standpoint, efforts are under way to use molecular markers in stool or serum for the detection of colon cancer. If the disease has substantial pathway heterogeneity, as suggested by the model in Fig. 1, combinations of markers spanning all routes to cancer are going to be needed for optimal sensitivity.

The biggest effect of the multiple pathways to colon cancer model relates to therapy. Despite decades of research, there continues to be uniformity in thinking of, and in treating colon cancer. This is poised to change (9). The different colon cancers have vastly different prognoses, ranging from favorable (MSI, BRAF unmutated) to very poor (CIMP, no MSI). There is increasing recognition of different responsiveness of MSI cases to 5-fluorouracil (which likely also extends to CIMP cases), and clinical trials will prospectively test treating these patients differently. Most recently, cases with KRAS mutations (most of which belong to the CIMP group) have been shown to have a very low response rate to adding cetuximab to chemotherapy. We are therefore approaching individualized therapy for colon cancer, whereby each tumor will have to be thoroughly molecularly profiled before therapy can be selected.

An important issue before translating these findings into clinical practice relates to technical measurement of the various

molecular events—methylation and CIN in particular. Different methods and different genes have led to substantially divergent estimates of CIMP frequency and its association with KRAS mutations. This is true for the study by Cheng et al., which, although they used an innovative methylation measurement technique, included genes that are not well associated with CIMP such as MGMT or APC (10). This may have led to an underestimation of the true frequency of CIMP. Estimates of CIN prevalence are also complicated by the method used and the purity of the samples (which affects loss of heterozygosity analysis to a greater extent than DNA methylation). Use of very sensitive technology (as was done here) results in very high estimates for CIN; these may not reflect reality as a certain background of LOH will happen with proliferation, in the absence of active mechanisms to promote genetic instability. A concerted effort by multiple investigators is needed to define the optimal methodologies, markers, and controls for defining the various colon cancer entities before the information can become truly clinically useful.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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