Letters to the Editor

Correspondence re: G. Anderson *et al.*, Intrachromosomal Genomic Instability in Human Sporadic Colorectal Cancer Measured by Genome-Wide Allelotyping and Inter-(Simple Sequence Repeat) PCR. Cancer Res., *61:* 8274–8283, 2001.

Letter

Anderson *et al.* (1) claim that cancer cells exhibit intrachromosomal instability on the basis of their finding thousands of "mutations" per cell among inter-SSR[1] repeats in polyps and carcinomas of the human colon and their absence in normal colonic crypts. Apparently this instability does not extend to mutation rates in selectable structural genes because most studies have found no such differences between normal and malignant cells in culture (2–5). Exceptions are found as expected among colon cancer lines deficient in DNA repair (6, 7), but four of five colon cancer lines without such deficiency did not yield high rates of spontaneous mutation (8). More recently, about 3.2 Mb of coding colorectal tumor DNA have been sequenced, and only three distinct mutations were identified (9). It was concluded that the accumulation of about 1 nonsynonymous somatic change/Mb tumor DNA is similar to that estimated for normal cells and suggests that most sporadic colorectal cancers do not display a mutator phenotype at the nucleotide level.

A much higher rate of gene amplification has been reported in malignant cells than in normal cells (10), which might be related in some way to the increased inter-SSR alterations. Gene amplification is associated with structural changes in chromosomes (11), and the latter occur with high frequency at early stages of human colorectal tumors (12, 13). The sequencing of colorectal cancer DNA, however, suggested that normal mutation rates coupled with selection are sufficient to explain tumor progression (9), as had previously been inferred for human tumors (14, 15) and spontaneous transformation in cell culture (16). It also suggests that most of the mutations in sporadic colorectal cancer start to accumulate in normal-appearing tissue, as had been indicated by genetic reconstruction of suitably informative colorectal cancers deficient in DNA repair (17).

Although low-level microsatellite instability occurs in most colorectal cancers, no association was found with any clinicopathological or molecular variable (18). Hence, the changes found in length of SSRs (1) are not representative of or correlated with functional mutations. The frequent slippage in copying simple repeats could be a response to the tumor microenvironment (18), but this remains to be determined.

Anderson *et al.* (1) seem to assume that genetic instability in cancers is a fact, when the most common form of functional mutation at the intrachromosomal level, namely, base substitution of structural genes, apparently occurs in the normal range (9). It is crucial for cancer research to identify just what is and what is not unstable in cancer cells. The evidence for chromosome instability in cancer is certainly compelling (12, 13), but the source of that instability may be in the microenvironment of the cancer cell (19, 20) as well as within it (21).

A missing ingredient in the discussion is the frequency of inter-SSR mutations in well-defined experimental systems. This has now been remedied with the report of the absence of genetic instability in 49 chemically induced thymic lymphomas, papillomas, and squamous cell carcinomas of mice (22). The implication of this finding is that the high frequency of inter-SSR mutations in human colorectal cancers results from the prolonged preclinical history of such tumors (17), and that this form of instability is not necessary for the neoplastic state.

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The abbreviation used is: SSR, simple sequence repeat.

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Reply

Rubin raises several issues. Classic work using *HPRT* assays of tumor and normal cell lines revealed no consistent tumor-specific changes in mutation rates for this gene (1, 2); from this, Rubin argues that structural genes are not mutated internally at elevated rates in tumor cells. However, as reviewed by Albertini (3), the *HPRT* system is not a broadly sensitive measure of genomic damage, instead identifying predominantly point mutants. Colorectal and other solid tumors, aside from those with microsatellite instability, are established to not have elevated point mutation rates; this does not mean that they do not exhibit other forms of intrachromosomal instability.

Rubin cites a report by Wang *et al.* (4) describing the sequencing of 3.2 Mb of DNA from 12 early-passage colorectal tumor cell lines; 320 changes were found as compared with published human DNA sequence data. Ninety of these represented known single-nucleotide polymorphisms; the remaining 230 were sequenced from corresponding normal tissue DNA, identifying three tumor-specific mutations that were assumed to have arisen before generation of the tumor cell lines. Three mutations per 3.2 Mb represents about 3,000 events/haploid tumor cell genome; this very rough calculation is based on only these three events. Wang *et al.* then calculated, from estimates of normal mutation rates, that somewhere between 600 and 18,000 events should have occurred, with 3,000 falling within this range. From this, they and Rubin conclude that intrachromosomal instability must occur rarely, if ever, in tumors without microsatellite instability. This conclusion cannot be justified based on the very broad range estimated for normal rates, the very small number of events found in the tumor cell line DNAs, and the extreme extrapolation. This is compounded by no actual measurement of how many events occurred in normal cells in the same amount of DNA.

Our report (5) eliciting Rubin's commentary described our use of the sampling technique of inter-(SSR[1]) PCR to measure genomic damage, comparing colorectal tumor DNA with normal tissue DNA from each patient. A previous report by us estimated the average number of intrachromosomal events/tumor cell genome to be at least 11,000 (6), although sampling considerations will raise this number higher (7). After this previous report, Rubin had again suggested that we were seeing events that had arisen at normal rates, with tumors representing a selective outgrowth of those cells containing sets of events that produce malignancy (8). He proposed that individual normal colonic crypts, clonal in origin, would reveal similar numbers of events to what we had seen in colorectal tumors. Thus we performed the experiments comparing DNAs from individual crypts, as reported in our *Cancer Research* article (see Fig. 7 in Ref. 5); this revealed no detectable genomic differences between individual normal crypts. Whereas genomic damage obviously will be occurring in normal colonic mucosal cells, it is below the threshold of detectability by inter-(SSR) PCR and thus well below the level measured in colorectal tumors.

Rubin fails to comprehend the basis of inter-(SSR) PCR. We are not examining the length of classical SSRs; by using short SSR-based primers anchored at the 3′ end, facing away from the repeat element itself, we are amplifying the region between repeats in those cases where the repeats are within a few kilobases of each other and in inverted orientation (9).

Intrachromosomal instability is becoming understood as new technologies come to be applied. Array-based comparative genomic hybridization shows the complexities occurring within amplicons, microdeletions, and other events at a level below the detection ranges of previous methodologies (10). *In situ* hybridization technologies reveal details as genomic instability generates cell-to-cell differences within the tumor cell population (11), and laser capture microdissection confirms the ensuing tumor heterogeneity (12). Yeast models of intrachromosomal instability and the finding of homologous genes involved in human familial cancers provide additional clues to understanding the underlying mechanisms involved in genomic instability in cancer (13–15). The emerging picture of adult-onset solid tumors as the highly evolved consequence of genomic destabilization, generating malignant cell populations of substantial genomic diversity, can be neither dismissed nor ignored.

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