Microsatellite Instability

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Microsatellites are stretches of DNA in which a short motif (usually one to five nucleotides long) is repeated several times. A typical mononucleotide-repeat microsatellite might be, for instance, a stretch of 13 adenines, abbreviated (A)\textsuperscript{13}. The most common microsatellite in humans is a dinucleotide repeat of cytosine and adenine, (CA)\textsubscript{h}, which occurs in tens of thousands of locations in our germ line. Trinucleotide repeats are prone to expansion in meiosis, and when abnormally expanded they lead to a variety of neurologic disorders (such as Huntington’s disease, the best known of these) and fragile chromosomal sites (such as the fragile X syndrome, one of the commonest forms of inherited mental retardation). When a microsatellite shows heritable and stable differences from person to person in the number of repeats it involves, it is said to be polymorphic. For instance, a CA microsatellite may have four alleles: (CA)\textsubscript{11}, (CA)\textsubscript{14}, (CA)\textsubscript{15}, and (CA)\textsubscript{20}. Polymorphic microsatellites of this type are excellent genetic markers and are important tools for mapping disease-causing genes by linkage, for performing investigations in forensic medicine, and for studying genetic deletions (loss of heterozygosity) in tumors.

Microsatellite instability is a situation in which a germ-line microsatellite allele has gained or lost repeat units and has thus undergone a somatic change in length. Because this type of alteration can be detected only if many cells are affected by the same change, it is an indicator of the clonal expansion that is typical of a neoplasm. Ten years ago, microsatellite instability was serendipitously discovered to be such a marker in hereditary nonpolyposis colorectal cancer (also called the Lynch syndrome). After susceptibility to hereditary nonpolyposis colorectal cancer had been mapped to a locus on chromosome 2 by linkage analysis, investigators expected to find a tumor-suppressor gene and searched for loss of heterozygosity among dinucleotide repeats in the critical genetic region. Instead, what was found in all the hereditary nonpolyposis colorectal cancers studied were microsatellite alleles that had changed in length as a result of nucleotide insertions or deletions. These modifications were found not only in microsatellites in the critical genetic region but also in microsatellites virtually everywhere in the genome of the tumor. This remarkable phenomenon was termed “replication error” and later renamed “microsatellite instability.”

It was soon realized that the widespread microsatellite instability in hereditary nonpolyposis colorectal cancers was associated with defective DNA-mismatch repair. Nucleotide mismatches occur normally when two strands of DNA replicate, but almost all such errors are quickly corrected by a molecular proofreading mechanism. Studies of hereditary nonpolyposis colorectal cancers revealed mutations in mismatch-repair genes such as MSH2 and MLH1, which encode proteins that repair nucleotide mismatches.

Defective mismatch repair presumably facilitates malignant transformation by allowing the rapid accumulation of mutations that inactivate genes that ordinarily have key functions in the cell. It seems that defective mismatch-repair genes, by failing to produce proteins that correct nucleotide mismatches during DNA replication, promote mutations in other genes. But genes carrying microsatellites in their own coding sequences are also involved. Two such genes are BAX and TGFBR2. BAX harbors a (G)\textsubscript{8} microsatellite in its coding region, and in about 35 percent of all tumors with defective mismatch repair, this (G)\textsubscript{8} microsatellite has lost one or two guanines, resulting in a frame shift that inactivates the BAX gene. (In a frame-shift mutation, the deletion or insertion of a nucleotide shifts the normal sequence of nucleotides in a triplet codon.) This alteration is believed to contribute to carcinogenesis by disrupting the apoptosis pathway mediated by Bcl-2. The TGFBR2 gene, which encodes transforming growth factor β (TGF-β) receptor II, harbors an (A)\textsubscript{10} repeat that undergoes a frame shift in up to 90 percent of all hereditary nonpolyposis colorectal cancers. This mutation leads to a disruption in the function of TGF-β, a tumor suppressor of prime importance in colorectal cancer. Many other genes with coding microsatellites have recently been shown to be mutated in mismatch-repair-deficient colorectal cancer, but their precise roles, if any, are not well understood.

One intriguing consequence of mutations caused by microsatellite instability pertains to the spectrum of tumors in hereditary nonpolyposis colorectal
Cancer. Carriers of mutations that predispose them to colorectal cancer also have greatly elevated risk of endometrial cancer and a moderately increased risk of ovarian and gastric cancer, but they are not at increased risk for lung, prostate, or breast cancer. Might this selectivity depend on whether or not the key genes involved in each type of cancer have microsatellites in their coding regions? Is the predisposition to particular kinds of cancer and to the molecular mechanism of deficient mismatch repair in large part due to altered microsatellites in the coding sequences of certain genes? As of yet, these questions have no answers.

Deficient mismatch repair occurs in approximately 15 percent of all colorectal cancers and can arise through either of two mechanisms (see Figure). In hereditary cases (e.g., hereditary nonpolyposis colorectal cancer), the cause is mutational inactivation of one of the three main mismatch-repair genes (MLH1, MSH2, and MSH6), leading to deficient mismatch repair and, ultimately, cancer. However, many colorectal cancers show microsatellite instability without evidence of germ-line abnormalities. In these cases, the cause is biallelic methylation of the promoter sequences of MLH1 — an epigenetic, not inherited, change that leads to a deficiency of mismatch repair.

Tumors exhibiting chromosomal instability and microsatellite instability resemble each other in all but a few distinct ways (see Figure). Tumors with chromosomal instability have mutations in TP53 and gross chromosomal abnormalities, whereas those with microsatellite instability have frame-shift mutations in specific target genes. The interesting contrast in survival between the two types of colorectal cancers remains unexplained. In this issue of the Journal, Ribic et al. (pages 247–257) provide new insight by showing that adjuvant chemotherapy with fluorouracil benefited patients with tumors exhibiting chromosomal instability, but not those with tumors exhibiting microsatellite instability.

Although the authors do not attempt to explain the molecular basis of this finding, their data suggest that fluorouracil-based adjuvant therapy actually decreased the rate of survival among patients with tumors exhibiting microsatellite instability. This is a noteworthy and apparently counterintuitive finding in view of the fact that tumors with microsatellite instability were associated with an overall better survival than those with chromosomal instability. The authors carefully urge readers not to alter their clinical practices until these data are confirmed. If the most commonly used adjuvant therapy does not benefit patients with tumors exhibiting microsatellite instability, we should consider testing all colorectal cancers for microsatellite instability.

Testing for microsatellite instability is straightforward. DNA from the tumor and from normal tissue (blood, a buccal smear, or normal colonic mucosa) is tested by genotyping fluorescently labeled polymerase-chain-reaction products with the use of an automated sequencer. A panel of five microsatellite markers is usually enough; microsatellite instability in two or more of them is a positive result. Such tests would serve at least two purposes. First, they could help physicians assess a patient’s prognosis and perhaps guide his or her therapy. Second, they could serve as a powerful method of screening for hereditary nonpolyposis colorectal cancer. It is safe to predict that much more will be written about microsatellite instability.

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