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REVIEW

Testing tumors for microsatellite instability

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The methods for determining microsatellite instability in tumors are highly heterogeneous. Recently a 5-marker panel of microsatellites was suggested for this purpose. In this review attention is drawn to the fact that microsatellite instability can be assessed by analyzing tumor DNA with a single marker, BAT-26, and that normal tissue DNA from the same individual needs to be analyzed only when an aberrant allele is seen in the tumor. Whilst this simple procedure does not distinguish between different types and degrees of instability, it should be sufficient for many purposes, such as screening colorectal cancers for mismatch repair deficiency.

Keywords: cancer; colorectal; microsatellite; instability; stability; hereditary; hereditary nonpolyposis colorectal cancer (HNPCC); replication error (RER); microsatellite instability (MSI)

A recent report[1] from the International Workshop on Microsatellite Instability and RER Phenotypes in Cancer Detection and Familial Predisposition, 8–9 December 1997, made recommendations regarding the testing and interpretation of microsatellite instability (MSI). In essence, a 'reference panel' of five microsatellites was recommended for future research in the field. Two important reservations were made. It was explicitly stated that the recommendations apply to colorectal cancer only. Furthermore, the text of the report states that other loci and panels may prove to be of equal utility.

Nevertheless it is possible that the recommendations contained in the report may be taken too literally; that is, that MSI determinations may be viewed as deficient or unreliable if the recommended marker panel is not used. The purpose of this brief review is to draw attention to the fact that, as shown recently, a far easier and less costly, but not necessarily less efficacious, method of testing for MSI in colorectal cancer should be considered for some purposes.

Because MSI is a hallmark of tumors caused by the inactivation of mismatch repair genes, and because some patients with MSI^+ tumors have germline mutations in mismatch repair genes, it is clinically relevant to screen for MSI followed by evaluation of the mismatch repair genes in the germlines of patients whose tumors are MSI^+. Thus new cases and families with the hereditary non-polyposis colorectal cancer (HNPCC) syndrome will be detected and may benefit from high-risk clinical screening for cancer and precancerous conditions. It is now technically possible to screen for HNPCC among newly diagnosed colorectal cancer patients, and a significant, but not necessarily unique role in such screening belongs to MSI testing. There may be a need for thousands or tens of thousands of MSI tests for this purpose.

The markers BAT-26 and BAT-25 that are both contained in the recommended panel[1] are single nucleotide tracts that show a single allele in the germline and normal somatic cells of most individuals.[2,3] In BAT-26 this consists of a run of 26 adenosines, $(A)_{26}$. Occasional individuals have 27, 25 or 24 As instead of 26. As these individuals represent less than 5% of the population, the BAT-26 marker has been termed quasi-monomorphic. The polymorphic nucleotide run in marker BAT-25 mainly consists of $(T)_7A(T)_{25}$. This allele is the most common; other alleles so far seen in normal individuals from the populations studied have either a loss of one nucleotide or an addition of one or two nucleotides.

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Both BAT-26 and BAT-25 are highly sensitive to MSI. In a first study of 160 colorectal cancer tumors and cell lines, the MSI status of 159 of these could be correctly determined by studying BAT-26 alone.[4] This conclusion was made after comparing the results of BAT-26 with those of a combined panel of a minimum of 31 other microsatellite loci. In an extension of these studies, BAT-26 was analyzed in a total of 542 solid tumors from various organs.[5] In 539/542 tumors BAT-26 identified microsatellite stability/instability concordant with the results from variable batteries of other microsatellite markers. In each case normal tissue was also studied. This allowed the quasi-monomorphic allele distribution of BAT-26 and the distribution of alleles in BAT-25 described above to be determined.

Recently, a prospective study of 509 consecutive, unselected, sporadic colorectal cancer patients was reported.[6] Using a battery of other microsatellite markers, 446/509 tumors were classified as MSI^- whilst 63/509 were MSI^+. When BAT-26 was studied in all tumors, it showed abnormal alleles in six of the 446 tumors previously classified as MSI^- and, further, showed abnormal alleles in 58 out of the 63 tumors previously classified as MSI^+. All 69 patients whose tumors were MSI positive for either the conventional markers or BAT-26 were subjected to mutational analyses by exon-by-exon sequencing of MSH2 and MLH1 from non-tumor DNA. Ten patients were germline mutation positive (new cases of HNPCC); all ten were MSI positive both with conventional markers and BAT-26.

The evidence available so far lends itself to the suggestion that colorectal tumors can be MSI classified by studying BAT-26 only. Notably, it is not necessary to study normal tissue at first. In all patients whose tumors show alleles other than $(A)_{26}$, normal tissue should be studied as well so as to determine whether the change is acquired or germline. In this way misclassification due to rare aberrant germline alleles can be avoided. Such alleles have not yet been seen, or at least not identified as germline variants,[5] but could well occur in other populations than the Caucasians who have been studied so far.

Available evidence shows that in MSI, BAT-26 always loses rather than gains As. So far, not a single tumor has been reported in which a gain of A has occurred. The typical loss in BAT-26 comprises 5–15 nucleotides and, remarkably, there is no overlap between rare normal alleles with $(A)_{25}$ or $(A)_{24}$ and MSI positive tumors with $(A)_{21}$ or less. A gray zone of $(A)_{22}$ or $(A)_{23}$ may exist. According to the data by Zhou *et al*[7] these cases can be resolved into either MSI^+ or MSI^- by studying BAT-25.

Until more data on the molecular background of MSI become available it is certainly recommendable to study many markers for various research purposes and the 5-marker panel recommended[1] is a useful one, eg for comparisons between laboratories. My proposal is meant for the practical situation where colorectal, and perhaps other, tumors need to be tested for MSI for the purpose of evaluating the patient for HNPCC. MSI screened for in this way (a single marker studied in tumor tissue only) should be sufficiently easy and sensitive for clinical studies of both high and low-risk patients. Together with data on family history and age of onset this test provides a reasonable means of pre-screening for HNPCC.

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