

## MINI-REVIEW: Microsatellite instability in human cancer

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Short tandem repeats of mono, di, tri or tetra nucleotide motifs occur frequently in eukaryotic genomes. These repeats are known as microsatellites. The most thoroughly characterized class of microsatellite consists of CA (or GT) repeats. CA repeats of at least 10 repetitions occur on average every 30-60 kb throughout the human genome, the total number being in the order of 100,000 (Weber and May, 1989; Litt and Luty, 1989; Stallings *et al.*, 1991). Trinucleotide repeats are less common, with the most abundant class, (AAT)<sub>n</sub>, occurring approximately once every 500 kb (Gastier *et al.*, 1995).

Microsatellites are inherently unstable due to the fact that DNA polymerases frequently slip while replicating such regions. This is readily observed when microsatellites are copied *in vitro* using the polymerase chain reaction. In addition to reaction products identical to the microsatellite being amplified, copies with different repeat lengths are also produced, the most common of which contain either one less or one more repeat motif than the starting sequence (Hauge and Litt, 1993). Such slippage also occurs during DNA replication *in vivo* although the cell's mismatch repair system corrects most resulting DNA mismatches and restores the original length. Nevertheless, it appears that replication errors occur at such a rate in microsatellites that the repair system cannot correct them all. As a result, mutations occur at microsatellite loci at the rate of  $10^{-4}$  to  $10^{-5}$  per division in mammalian cells (Farber *et al.*, 1994). If the mismatch repair system is defective, the rate of mutation increases 100-1000-fold (Strand *et al.*, 1993; Shibata *et al.*, 1994; Heale and Petes,

1995). When microsatellite mutation rates reach these levels, "microsatellite instability" (MSI) is said to occur and the cell line in which it occurs is said to exhibit a replication error (RER<sup>+</sup>) phenotype. In man, the RER<sup>+</sup> phenotype and MSI have been associated with cancer.

Since MSI occurs in tumors and not in normal tissue it is readily detected by comparing arbitrarily selected microsatellite loci in DNA extracted from normal and tumor tissues. In tumors exhibiting MSI, additional alleles are detectable that are not present in corresponding normal DNA. Tumor associated MSI was first observed in colon cancer, particularly in individuals from families with autosomal dominant non-polyposis colorectal cancer or HNPCC (Aaltonen *et al.*, 1993; Thibodeau *et al.*, 1993; Ionov *et al.*, 1993). This observation provided an important clue to the nature of the inherited mutated gene(s) responsible for HNPCC, since the genes responsible for the mismatch repair system became natural candidates. Indeed, subsequent studies resulted in the identification of four human genes encoding proteins involved in DNA mismatch repair, each of which was mutated in the germline of some HNPCC families (Fishel *et al.*, 1993; Leach *et al.*, 1993; Papadopoulos *et al.*, 1994; Bronner *et al.*, 1994; Nicolaides *et al.*, 1994). The genes are *hMSH2*, *hMLH1*, *hPMS1* and *hPMS2* and are mutated in the germline of approximately 60%, 30%, 5% and 5%, respectively, of HNPCC kindreds. The proof that these mutated genes are indeed responsible for colorectal cancer comes from the observation that in tumor tissue the normal (non-mutated) allele is inactivated either by a somatic mutation or reduction to homozygosity (Leach *et al.*, 1993; Nicolaides *et al.*, 1994; Lynch *et al.*, 1994). The observation that four different genes can be responsible for the same form of inherited predisposition to colorectal cancer accounts in part for its relatively high frequency affecting as many as 1 in 200 individuals in the Western world (Lynch *et al.*, 1993).

Following the demonstration that MSI is a feature of the colorectal tumors that occur in HNPCC families, it was found that the same phenomenon can be observed in benign and premalignant lesions in the same patients, indicating that MSI is an early event in tumor progression (Aaltonen *et al.*, 1994; Suzuki *et al.*, 1994). Thus, it was clearly established that the mismatch repair proteins function as tumor suppressor genes which when mutated lead to the rapid accumulation of further mutations and malignancy. The very high mutation rate in cells with the RER<sup>+</sup> phenotype resulting from loss of mismatch repair function has been cited as evidence for the existence of a mutator phenotype in human cancer. Such a mutator phenotype had previously been hypothesized to account for the occurrence of multiple mutations in tumors despite the relatively low rate of mutation that occur in normal human cells (Loeb, 1991, 1994).

The general relevance of MSI to human cancer was subsequently established by showing that this phenomenon can also be detected in approximately 30% of sporadic colon cancers (Lothe *et al.*, 1993; Liu *et al.*, 1995) as well as 5-30% of cases of brain (Dams *et al.*, 1995), oral (Ishwad *et al.*, 1995), esophageal (Nakashima *et al.*, 1995), lung (Ryberg *et al.*, 1995), breast (Contegiacomo *et al.*, 1995), gastric (Lin *et al.*, 1995), pancreatic (Brentnall *et al.*, 1995), renal (Uchida *et al.*, 1994), prostate (Egawa *et al.*, 1995), bladder (Zulueta *et al.*, 1993), endometrial (Duggan *et al.*, 1994) and uterine (Mitra *et al.*, 1995) cancers (N.B. single recent references are cited for each cancer type as examples and as sources for the extensive literature concerning the incidence of MSI in diverse types of human cancer). With the exception of work with colon cancer none of the other studies of MSI included the laborious task of searching for mutations in one of the four mismatch repair genes. Thus it remains to be formally proven that MSI is caused by mutations in these genes in all cases. Nevertheless, there is no doubt that MSI is a widespread phenomenon associated with multiple forms of human cancer.

A crucial question is whether the mutations in microsatellites observed in RER<sup>+</sup> cells could themselves be responsible for carcinogenesis. Repetitive regions of the genome such as microsatellites have, in the past, been thought to represent "junk" DNA. It is now apparent, however, that alterations in such sequences can be causally related to human disease. The most clear cut examples include various inherited neurodegenerative diseases such as Huntington's Disease, Fragile X Syndrome and Myotonic Dystrophy (Richards and Sutherland, 1994). In these disorders, trinucleotide repeats are greatly expanded in the appropriate disease gene to the point where they

become highly unstable and disrupt gene function (Hoffman and Jaffurs, 1993). There is no known association between trinucleotide expansion disorders and cancer and no evidence that such massive microsatellite expansions occur in tumors with MSI. Nevertheless, there are other mechanisms whereby microsatellite alterations can directly alter gene expression and protein function. Many coding regions contain very short runs of mononucleotides and dinucleotides that can be considered as extremely small (or mini) microsatellites. Analysis of mutations in the marker gene *HPRT* in colorectal carcinoma cell lines with MSI showed that a run of 6 guanines was frequently mutated to 7 such nucleotides thus disrupting the reading frame (Bhattacharyya *et al.*, 1995). Similar mutations were found to be extremely rare in cell lines not exhibiting MSI. The first reported example where this kind of mutation was implicated in cancer progression came from studies of the TGF- $\beta$  receptor (Markowitz *et al.*, 1995). TGF- $\beta$  (transforming growth factor- $\beta$ ), despite its name, actually inhibits growth of epithelial cells. It had been known for some time that several types of cancer cells, as they become more malignant, often stop responding to TGF- $\beta$ , thus becoming impervious to its natural inhibition of cell growth (Fyfan and Reiss, 1993). The basis of reduced responsiveness to TGF- $\beta$  in colon cancer was traced to the loss of the RII subunit of the TGF- $\beta$  receptor and this was found to be a common occurrence in cell lines and tumors exhibiting MSI (Markowitz *et al.*, 1995). The underlying mutations proved to be due to the loss of one or two adenines from a run of ten such nucleotides in the 5' half of the RII subunit gene which resulted in the synthesis of truncated receptor proteins.

The observation of mutated "mini-microsatellites" within genes responsible for tumor progression neatly brought the study of MSI into human cancer full circle. Original observations of MSI in tumors led to the demonstration that hereditary mutations in genes encoding components of the mismatch repair system were responsible for HNPCC. Such mutations were found to also occur in sporadic tumors and MSI demonstrated to be a widespread phenomenon associated with human cancer. Finally, we now know that MSI itself can play a direct role in the disease. Less than three years have passed since the original description of MSI in human cancer. Progress has been breathtaking and there is no reason to believe that continued studies of this phenomenon will not only produce further insights into the molecular genetics of human cancer but also possibly new therapeutic and diagnostic approaches for this complex and devastating disease.

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