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Correspondence

Biological Significance of Microsatellite Instability-Low (MSI-L) Status in Colorectal Tumors

To the Editor-in-Chief:

Hawkins et al[1] claim that our demonstration of low levels of DNA microsatellite instability in neoplasms developing in hyperplastic polyps could be based on the use of "tetranucleotide repeat markers and MYCL markers." They add, "The biological significance of the microsatellite low instability phenotype is uncertain, and the definition of this phenomenon remains both arbitrary and subject to considerable operational flexibility."

Several groups have shown that when colorectal cancers are tested with a panel of microsatellite markers, cancers showing microsatellite instability are distributed bimodally with a breakpoint at around 30%.[2–4] Cancers low in microsatellite instability lack instability at mononucleotide repeats, for example BAT25, BAT26, and BAT40,[2] and rarely show loss of expression of DNA mismatch repair genes hMLH1, hMSH2, or hMSH6.[5,6] The separation of microsatellite instability-low and -high cancers is not arbitrary. Furthermore, the failure to separate these groups confounds the molecular characterization of cancers with the microsatellite-high phenotype. This will remain a problem until proper use of well-validated mononucleotide and dinucleotide markers is instituted.[7]

With regard to MYCL, our published experience dates back to 1995[8] and the marker has been well validated by others.[2] This polymorphic microsatellite marker has been sequenced and tracked within families to show that it is normally stable. In the context of DNA microsatellite instability, MYCL is highly mutable and sensitive for high and low levels of instability in both sporadic and familial colorectal cancers. Interpretation is straightforward and the marker gives reliable results in DNA extracted from formalin-fixed tissues. The reason for the high rate of mutability of this locus is unknown. However, it is incorrect to label MYCL as a tetranucleotide marker. It is a compound or complex marker comprising a mononucleotide repeat sequence that is flanked by tetranucleotide and pentanucleotide repeats (unpublished data). Although we often illustrate MYCL bandshifts in our publications, we do not rely on MYCL exclusively to test for MSI but employ a comprehensive panel of dinucleotide markers and mononucleotide markers. In a recently published series of adenomas from subjects with hereditary non-polyposis colorectal cancer, MYCL was the most sensitive marker for microsatellite instability-high adenomas but was insensitive for microsatellite instability-low adenomas, which were detected by the dinucleotide markers D2S123, D5S346, D10S197 and D18S58. Most of these adenomas showed loss of expression of either hMSH2 or hMLH1 concordant with the germline status of the individual.[5] It is not sensible to doubt the biological significance of microsatellite-low status in the face of this evidence and the suggestion that the use of MYCL accounts for our discrepant results is groundless.

The indisputable fact remains that a subset of colorectal polyps and cancers shows mutation in DNA microsatellite loci with (usually) dinucleotide repeats, albeit at a low frequency. In our experience and that of others, the microsatellite-low subset of sporadic cancers shows a high frequency of K-ras mutation.[9,10] K-ras mutation is commonly demonstrated in aberrant crypt foci and hyperplastic polyps.[11,12] These lesions may also show microsatellite instability to a low and sometimes a high level.[13,14] A higher incidence of microsatellite instability is observed in mixed polyps and serrated adenomas.[14,15] On the basis of these observations, we suggested that DNA microsatellite instability-low cancers might originate within the spectrum of serrated polyps (hyperplastic polyps, admixed polyps, and serrated adenomas).[15] We did not state that such a pathway would apply exclusively to microsatellite instability-low cancers.

Over the last 5 years, we have tested 33 cancers from 22 subjects with hyperplastic polyposis. Fourteen are microsatellite stable, 5 show low level microsatellite instability, and 14 show high level microsatellite instability. Our findings in 18 of the cancer samples have been published,[6,16,17] findings in an additional eight cancers are in press,[18] and unpublished results account for the remainder. Two of the microsatellite instability-high cancers were from an individual considered to have hyperplastic polyposis and hereditary non-polyposis colorectal cancer.[17] A germline mutation has not been found in this individual and the affected family could be an example of familial hyperplastic polyposis.[16]

It is evident from our data, and those of others,[14] that the serrated pathway of colorectal neoplasia is heterogeneous. The single case report by Hawkins et al[1] corroborates our findings and reiterates views that we havepublished and presented previously. We reject their claims that our results reflect the inappropriate use and overinterpretation of alterations in the microsatellite locus MYCL.

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Authors' Reply:

We are pleased to respond to the letter of Jass et al regarding our study of the genetic events underpinning colorectal cancer in a case of hyperplastic polyposis.[1] In that study, we did not find microsatellite instability in any of the hyperplastic polyps that we examined. We felt it relevant to contrast this finding with those of Jass and colleagues, who, as they point out in the letter above, have found instability in both polyps and carcinomas in the course of several studies of hyperplastic polyposis. Unfortunately, Jass and colleagues have taken exception to two comments made in this context.

Firstly, we state, "The use of different microsatellite markers and, in particular, the use of tetranucleotide repeat markers *and* the MYCL markers favored by Jass may contribute to the differences reported here."[1] (The italics are ours.) This is a simple statement without artifice. It is widely accepted that different microsatellites show different levels of instability, and we entirely accept the possibility that had we tested our patients' tumors using different markers, we might have found instability. The simple fact is that the MYCL marker is often used by Jass et al, but was not used by us in this study. Since, as Jass states above, "MYCL is highly mutable and sensitive for high and low levels of instability in both sporadic and familial colorectal cancers," we find no reason to retreat from our statement that differences in its use may have led to apparent differences in instability status. Importantly, we would emphasize that at no point in our article did we claim, as erroneously stated above, that the results of Jass "reflect the inappropriate use and overinterpretation of alterations in the microsatellite locus MYCL."

In the next sentence we state, "The true biological significance of MSI-L is uncertain, and the definition [of MSI-L] remains both arbitrary and subject to considerable operational flexibility." Again, Jass et al have found reason to contest this opinion vigorously, and this provides a welcome opportunity to consider this interesting issue in greater detail.

As stated by Jass and colleagues above, it is clear that a small percentage of tumors show a degree of instability at microsatellite loci that falls short of the level (two or more of five markers) proposed by the NCI working party[2] as clear evidence of instability. To separate them from microsatellite stable (MSS) tumors, these lesions have come to be known as MSI-L. It is not the existence, but the biological significance of this MSI-L group, that is at issue in this debate.

In their letter, Jass et al argue that MSI-L tumors are distinct from MSI-H tumors. This is a well recognized fact that underpins the "two of five" criteria provided by the NCI working group, and one with which we clearly agree. More importantly, Jass et al also reaffirm their view that MSI-L tumors are biologically distinct from MSS tumors. This is an important hypothesis that they have rightly pursued. To support this argument they state that MSI-L tumors show a higher frequency of K-*ras* mutation, and that MSI-L is more common in serrated neoplasms.

The alternative view, raised indirectly by our article and one roundly rejected by: Jass et al in their letter above, is that MSI-L tumors are not a biologically distinct subset. We would argue that there is also considerable evidence to support this viewpoint. Three issues are of particular relevance.