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Improved Microsatellite Instability Detection and Identification by Nuclease-Assisted Microsatellite Instability Enrichment Using HSP110 T17

To the Editor:

Microsatellite instability (MSI)¹ is characterized by the accumulation of mutations (insertions or deletions of several nucleotides) in microsatellites, caused by DNA mismatch repair system deficiency. Approximately 15% to 20% of colorectal cancers (CRCs) present with MSI, the origin of which can be sporadic or owing to genetic predisposition to Lynch syndrome/hereditary non-polyposis CRC or constitutional mismatch repair deficiency syndrome. The MSI phenotype is associated with a better survival rate for patients with stage II/III CRC and is also a major predictive biomarker for the efficacy of immune checkpoint blockade therapy in metastatic CRC. The gold standard method for MSI detection includes standard PCR followed by fragment analysis using the Bethesda or the Pentaplex panel, each composed of 5 microsatellite markers. However, this approach can lack analytical sensitivity, thus limiting potential clinical applications.

Recent attempts to improve the analytical sensitivity of MSI detection include a method based on mutation enrichment during *E-ice*-COLD-PCR. This method uses HSP110 T17 (HT17), which, compared with the Pentaplex panel, is an

improved microsatellite biomarker for the diagnosis of MSI/MSI-High in CRC and also bears prognostic and predictive information on the response to chemotherapy (1). However, it requires the use of an identification table to identify the mutations because a shift of HT17 microsatellite profiles is induced in wild-type (WT) and mutated samples because of the enrichment of stutter peaks during *E-ice*-COLD-PCR (1).

Here we describe a new approach for the detection and identification of MSI using HT17 and based on nuclease-assisted microsatellite instability enrichment (NaMSIE), which allows enrichment of MSI before PCR without causing a shift in microsatellite profiles. This method is a modified version of 2 recently published approaches for the sensitive detection of point mutations [no denaturation nuclease-assisted minor allele enrichment with probe overlap (ND-NaME-Pro)] (2, 3) and epimutations [methylation-specific NaME (MS-NaME)] (4), in which either genomic DNA or bisulfite-converted DNA is mixed with probes complementary to WT regions of interest in the presence of double-strand-specific DNA nuclease (DSN) (2, 4). The DSN then preferentially digests fully matched over mismatch-containing double-stranded DNA, allowing the enrichment of mutant and epimutant alleles (2, 4).

In NaMSIE, 2 partially overlapping probes, complementary to HT17 WT alleles (T17 and T16), were designed so that the mutant alleles, i.e., T15 to T10, induced 1 to 6 nucleotide mismatches, respectively (see Fig. 1 in the Data Supplement that accompanies the online version of this letter at <http://www.clinchem.org/content/vol64/issue8>). Contrary to (ND)NaME-PRO (2, 3) and MS-NaME (4), in which the mismatches are located in the center of the probes, the mismatches induced by the mutant alleles were located at the 3' end of the probes, as the presence of

2 WT HT17 alleles prevented encompassment of the microsatellite. Because of the presence of a poly-T repeat in HT17, the probes included several locked nucleic acid nucleotides to limit their length to 25 or 26 nucleotides and ensure a melting temperature (T_m) of 64 °C while the complementary overlapping region displayed a $T_m < 50$ °C (Exiqon LNA™ Oligo T_m Prediction Tool).

Optimized NaMSIE conditions included the preparation of the reaction mix on ice using 20 ng of DNA, 1× DSN buffer, 20 nmol/L of both LNA probes and 0.2 U of DSN (Evrogen) in a final volume of 11 μ L, followed by 20-min incubation at 68 °C and 2-min DSN inactivation at 95 °C. Next, 3 μ L of enriched product was used for PCR and fragment analysis. Fig. 1 in the online Data Supplement shows the HT17 profiles of samples either untreated or treated with NaMSIE; the WT sample presented no variation in its HT17 profile after NaMSIE, whereas the mutated samples (Co-115, T10/T11; LoVo, T12/T12; HCT-15, T13/14; SNU-1, T15/T15) presented a strong enrichment of MSI that was more pronounced for large deletions. The mutated alleles were easily identifiable after NaMSIE, contrary to *E-ice*-COLD-PCR (1). The analytical sensitivity was evaluated at 0.5% for Co-115, LoVo, and HCT-15 and at 2.5% for SNU-1. Twenty to 100 ng of DNA could be efficiently used as input DNA with NaMSIE, whereas lower amounts of DNA could abolish MSI enrichment because of the digestion of both mutated and WT alleles. NaMSIE was also evaluated on 8 DNA mismatch repair system deficiency CRC samples with a low level of tumoral cell content (5%–15%) and allowed the detection and identification of MSI in all samples (see Fig. 1 in the online Data Supplement).

The recent discovery of MSI in 14 types of cancer, as well as the demonstration that MSI can predict the efficacy of immune checkpoint

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¹ Nonstandard abbreviations: MSI, microsatellite instability; CRC, colorectal cancer; HT17, HSP110 T17; WT, wild type; NaMSIE, nuclease-assisted microsatellite instability enrichment; ND-NaME-PRO, no denaturation nuclease-assisted minor allele enrichment with probe overlap; MS-NaME, methylation-specific NaME; DSN, double-strand-specific DNA nuclease; T_m , melting temperature.

blockade therapy in solid tumors, highlights the need for analytically sensitive tools for clinical MSI diagnosis. Very recently, NaME-PRO has been evaluated for MSI detection using the Pentaplex panel (5). NaMSIE is an effective method for MSI detection with many potential clinical applications.

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Remediation of IgG4 Cross-Reactivity in IgG1 and IgG2 Subclass Assays

To the Editor:

We congratulate van der Gugten and colleagues on their publication identifying spurious IgG subclass (IgGSc)¹ results in patients with IgG4-related disorders (IgG4-RD) (1). Their observations provide the opportunity for in vitro diagnostics (IVD) manufacturers to improve the performance of routine clinical chemistry assays. Measurement of

IgGSc is widely used in the investigation of pediatric patients with suspected immunodeficiencies. Since 2001, IgG4 measurement has been recommended for the assessment of IgG4-RD (2), and physicians often coincidentally order the accompanying IgGSc assays. The observations by van der Gugten et al. present a challenge to IVD manufacturers; IgG4-RD is rare, and at normal serum concentrations there is no apparent cross-reaction. Previously, Ladwig et al. (3) reported good correlations between IgGSc concentrations by nephelometry and LC-MS/MS. These contrasting reports may result from variations in IgGSc measurements by LC-MS/MS, but most likely represent the different patient populations analyzed. While cross-reaction is not evident in normal or pediatric patients, the results presented by van der Gugten offer an opportunity for assay remediation.

We sought to identify IgG4 cross-reactivity in our IgG1 and IgG2 Optilite[®] assays in accordance with CLSI standard EP07-A2. Briefly, purified polyclonal IgG4 was supplemented at either 2 or 4 g/L with the appropriate phosphate-buffered saline diluent control into pooled normal sera containing lower reference range limit concentrations of IgG1 and IgG2 (approximately 3.8 g/L and approximately 2.5 g/L, respectively). IgG1 and IgG2 antisera were affinity adsorbed against polyclonal IgG4 purified from normal human sera and immobilized onto Sepharose; the resulting antisera were reassessed with the method described above (Fig. 1). Postaffinity adsorption recognition of polyclonal IgG4 was significantly reduced ($P < 0.0001$ for both IgG1 and IgG2 assays). However, the supplement recovery indicated that there remained a small, statistically significant dose-dependent difference between the base and supplemented pools for IgG2 ($P = 0.0013$) but not for IgG1 ($P = 0.1995$), and all results lay within

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¹ Nonstandard abbreviations: IgGSc, IgG subclass; IgG4-RD, IgG4-related disorder; IVD, in vitro diagnostic.