NEW MARKER PANEL FOR MICROSATELLITE INSTABILITY ANALYSIS IN HNPCC AND SPORADIC GASTROINTESTINAL CANCERS

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BACKGROUND

The microsatellite instability (MSI) phenotype is a hallmark of the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome as well as approximately 15% of sporadic colorectal (CRC) and gastric cancers. (*Altonen LA et al., Science 1993)*

MSI is an useful tool in identifying patients with HNPCC and sporadic CRC with defective DNA mismatch repair (MMR) genes. Moreover, the assessment of MSI status may be useful in establishing the oncological outcome of CRC patients and also in predicting tumor response to chemotherapy.

In 1997, a reference panel of 5 markers was suggested for MSI testing by the National Cancer Institute aiming to standardize the test.

(Boland CR et al., Cancer res. 1998), however this panel has limitations resulting from the inclusion of dinucleotide markers, which are less sensitive and specific for detection of tumors with MMR deficiencies compared to other markers that are currently available.

Based on the number of microsatellites found to be unstable, tumors are classified as stable, with low instability (MSI-L) and with high instability (MSI-H).

MISCLASSIFICATION OF MSI-H TUMORS DUE TO USE OF DINUCLEOTIDE REPEATS

Dinucleotide repeats in the aformentioned panel generally show instability in only 60%–80% of MSI-H tumors. *(Sutter C et al., Mol Cell Probes 1999)*

Dinucleotide repeat are also highly polymorphic, and their use in MSI screening of tumor DNA requires the analysis of corresponding germline DNA.

The interpretation of size alterations in dinucleotide repeats is difficult and can lead to misclassification. *(Loukola A et al., Cancer Res 2001.)*

Some MSI tumors with MMR deficiency caused by *hMSH6* mutation do not show alteration in dinucleotide repeats. *(Akiyama OY et al., Cancer Res 1997)*

BAT-26 IS SUFFICIENT FOR DETECTING THE MSI PHENOTYPE

Analysis of mononucleotide repeats BAT-25 and BAT-26 is sufficient to establish MSI status without reference to the germline DNA, because these markers are quasi-monomorphic. *(Hoang JM ET AL.; Cancer Res 1997),* however

- stability in BAT-26 locus is strongly evocative for the presence of wide intragenic deletion in the *MSH2* gene *(Pastrello C et al., EJHG 2006)* and
- polymorphic BAT-25 and BAT-26 alleles have been identified in 18.4% and 12.6%, respectively, of Afro-Americans *(Peruco M et al., Cancer Res 1999)* and in a small percentage of Caucasian individuals. *(Sood AK et al., Cancer Res 2001)*

AIM

To evaluate alternative loci as the most sensitive and specific markers for detection of tumors with defects in MMR and the identification of an optimal panel of markers for MSI-H detection.

PATIENTS AND METHODS

DNA Sample

Germline and tumor DNA was obtained from a prospective group of 443 CRC patients, who underwent surgery at the University Hospital of Padua (Italy) between 2003-2004. MSI status was previously determined following the Bethesda panel for MSI testing.

Multiplex PCR

After a careful search of medical literature, a set of new markers for a MSI Multiplex PCR approach (CC-MSI 04-61, AB ANALITICA s.r.l, Padova, Italy) was selected, which included ten mono-di nucleotide markers including the Bethesda panel (BAT-25, BAT-26,D2S123, D5S346, D17S250) and five additional markers (NR-21, NR-24, BAT-40, TGF-BetaR and D18S58). In addition, two tetranucleotide markers were added to identify sample mix-ups and/or contaminations.

Data Analysis

CRC were classified using the new panel and compared with previous results obtained using the Bethesda panel combined with immunohistochemical (IHC) analysis of MLH1, MSH2, MSH6.

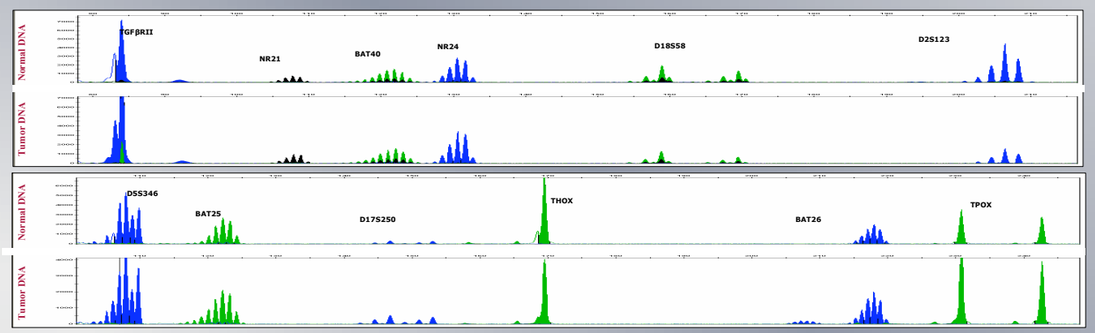
Acknowledgments

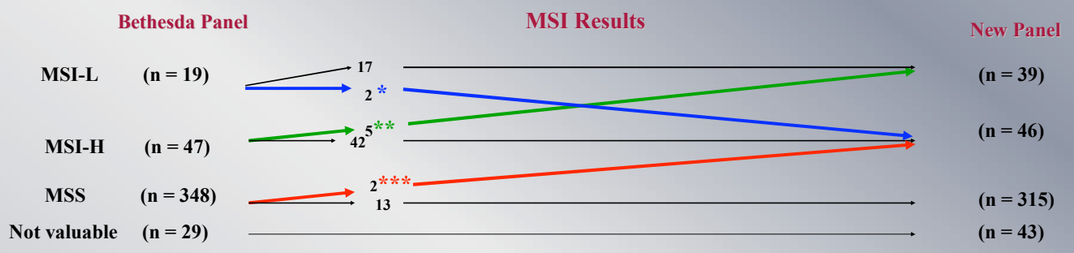
We are grateful to Miss Pizzini S. for support of laboratory analysis. This study, was partially supported by grants from the Regione Veneto (Azione Biotech 2), the Italian Ministry of Health (Programma speciale ex. art.12, 2004) and the Italian Ministry of Education, University and Research (MIUR: n° 3933).

rev170407

RESULTS

		Study Group, 443 patients		
MSI Test *Bethesda panel/ New Panel*	High	47/ 46		
	Low	19/ 39		
	Stable	348/ 315		
	n.v	29/ 43		
MSI-H *Bethesda panel*	BAT 26	39 (83%)		
	> 2 Dinucleotide	5 (10%)		
MSI-L *Bethesda panel*	BAT26	3 (16%)		
	Dinucleotide	16 (84%)		
IHC: no protein expression (Bethesda panel)		MLH1	MSH2	MSH6
MSI-H		19	7	10
MSI-L		2	0	0





Features of patients who shifted MSI category

MSI-L * → MSI-H ▪ Instability BAT26 ▪ Mucinous aspect and colon DX ▪ IHC: hMLH1 absent

MSI-H ** → MSI-L ▪ dinucleotide instability ▪ Colon sx-rectum and no mucinous ▪IHC MMR: present ▪NO mutations MMR genes

MSS *** → MSI-H ▪ Instability BAT40, NR21, NR24 ▪ Mucinous aspect and colon dx ▪ IHC MMR: present

Compared to the MSI testing obtained with the Bethesda panel, the results using the new panel showed that 5 MSI-H (at only dinucleotides markers) shifted to MSI-L category, 2 MSI-L cases (at only BAT 26 mononucleotide) shifted to MSI-H category and 2 stable tumors shifted to MSI-H. Overall 10 cases shifted from one cathegory to the other using the new panel for MSI testing.

Absence of MMR protein expression at IHC was found in 30/46 (65%) tumors with MSI-H using the new panel, whereas it was found in 27/47 (57%) cases using the Bethesda panel.

CONCLUSIONS

1. MSI-H status resulting from the instability of dinucleotide markers seems to be an almost certainly microsatellite stable phenotype;
2. A higher percentage of MSI-H (using the new panel) shows absence of MMR protein expression as compared to MSI-H tumors as defined using the Bethesda panel.
3. The new panel used in this study seems to be promising in defining MSI status of CRC

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