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No evidence for microsatellite instability in acute myeloid leukemia

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Microsatellite instability (MSI) is the somatic acquisition or loss of bases within repetitive DNA sequences due to defects in DNA mismatch repair, and is associated with an increased overall mutation rate. MSI is a key feature of certain solid tumors, particularly adenocarcinomas of the endometrium (found in approximately 30% of patients), colon (15%

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AUTHORSHIP CONTRIBUTIONS

CJW, A-KE, RB, AdlC and CDB contributed to the study design; CJW, A-KE, RB, AdlC and CDB contributed to the data interpretation; CJW, A-KE, RB, KM, AdlC and CDB wrote the manuscript; CJW, A-KE, LKG and MB performed laboratory-based research; JK performed statistical analysis; AJC, KM, JEK, BLP, ESW, RMS and CDB were involved directly or indirectly in the care of patients and/or sample procurement. All authors read and agreed on the final version of the manuscript.

CONFLICT OF INTEREST

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of patients) and stomach (15% of patients), and is also less frequently observed in other tumor types.[1] In colorectal cancer, the presence or absence of MSI is important because MSI-positive (MSI^+) patients exhibit superior outcome compared with microsatellite stable patients.[2] Conversely, MSI has been shown to associate with poor outcome in specific subsets of endometrial cancer patients.[3] Recently, newly developed immune checkpoint inhibitors have shown substantial promise as therapies for patients with MSI^+ solid tumors, and are also currently being explored for treatment of hematologic malignancies.[4]

Acute myeloid leukemia (AML) is characterized by the presence of both cytogenetic and molecular genetic abnormalities, and AML genomes tend to have far fewer somatic mutations than most solid tumors.[5] Even though the absolute number of mutations in AML patients is generally small, it has been shown that an increased number of somatic mutations associates with inferior outcome.[6] Thus, MSI status might potentially have prognostic implications for AML. The existence and importance of MSI in AML has previously been examined in several studies[7–9] but the results are still controversial. While some studies report MSI to be completely absent, others report that as many as 20 percent of *de novo* AML cases exhibit MSI.[7–9] These reports typically examined relatively small patient cohorts (<100 patients), and tested for MSI using the conventional method of fluorescence polymerase chain reaction (PCR) of a small panel of microsatellites followed by capillary electrophoresis. To our knowledge, the largest cohort of AML patients previously examined for MSI was 132 patients.[8]

In recent years, it has become more common to detect MSI by next-generation sequencing (NGS), because this method not only allows greater numbers of microsatellites to be assessed compared with conventional MSI screening, but also can be performed more efficiently.[10] Herein, we for the first time applied NGS-based MSI detection to AML in what we believe is the largest AML cohort screened for MSI to date. Among the 1394 AML cases in this study, we failed to find even a single case that exhibited MSI, and conclude that MSI is either exceedingly rare or completely non-existent in adult *de novo* AML at diagnosis.

We first examined MSI status in a cohort of 1371 AML patients, comprised of 1364 cases with *de novo* AML and seven with therapy-related AML (t-AML). As part of a separate mutation discovery study,[11] all cases were sequenced for 80 genes using an amplicon-based deep sequencing panel. To detect MSI we used a method similar to mSINGS[10] combined with a variant caller we previously developed for examining mutations in microsatellites.[12] Briefly, microsatellites contained on the target panel were manually identified using the manifest file, and 18 well-covered microsatellites without changes larger than one monomer present in germline DNA were selected for investigating MSI. For each sample and each microsatellite, the number of reads containing different length repeats was calculated using a custom script. Repeats that were represented in 5% of reads or greater were counted as alleles, and the total number of alleles for each sample was calculated. Samples with greater than three standard deviations from the mean of alleles were flagged as possibly displaying microsatellite instability. In order to be considered MSI^+ using mSINGS, a sample must display elevated allele counts (the products of somatic strand-slippage mutations associated with MSI) at >20% of microsatellites (in this case at least 4 out of 18).[10] None of the cases we examined met this criterion for being considered MSI^+. One sample had elevated allele

counts at two microsatellites, 67 samples were significantly elevated for one microsatellite and 1303 samples did not have elevated allele counts for any of the microsatellites (Table 1).

To more precisely examine MSI status, we focused on the subset of 86 AML patients with available germline DNA specimens (57 from complete remission samples and 29 buccal swab samples taken at diagnosis). The distributions of microsatellite lengths for each pair at the 18 microsatellites was assessed for significant differences between the AML and germline DNAs. Seventy-nine showed no differences at any microsatellite, as seen for a representative sample in Figure 1a. For eight pairs there were small but significant differences between the leukemic and germline DNAs. However, subsequent examination of these eight cases using the gold-standard multiplexed microsatellite PCR analysis system (Promega, Madison, WI) proved that none of these cases were MSI^+ (Figure 1b).

Because MSI^+ tumors also have elevated somatic mutation rates in non-repetitive DNA sequences, resulting in 10–100 times as many total mutations as in microsatellite stable tumors,[4,13] we next examined the total non-synonymous mutation counts for the entire ~250kbs covered by the target panel. Most samples (80%) had 1–4 mutations and the two most highly mutated samples had only 9 mutations each (Figure 1c). Since any putative MSI^+ samples would have tens if not hundreds of mutations, these data support the complete absence of MSI.

Finally, because it has been proposed that t-AML might be more prone to MSI,[7,14,15] we investigated the existence of MSI in an additional 23 t-AML cases using microsatellite PCR and capillary electrophoresis. No samples showed instability at any of the microsatellites we examined, consistent with MSI being rare or non-existent in t-AML (Supplementary Table S1).

In summary, we did not find any MSI^+ AML samples. The absence of even a single MSI^+ case within the relatively large cohort we examined provides strong evidence that MSI is non-existent in adult *de novo* AML at initial diagnosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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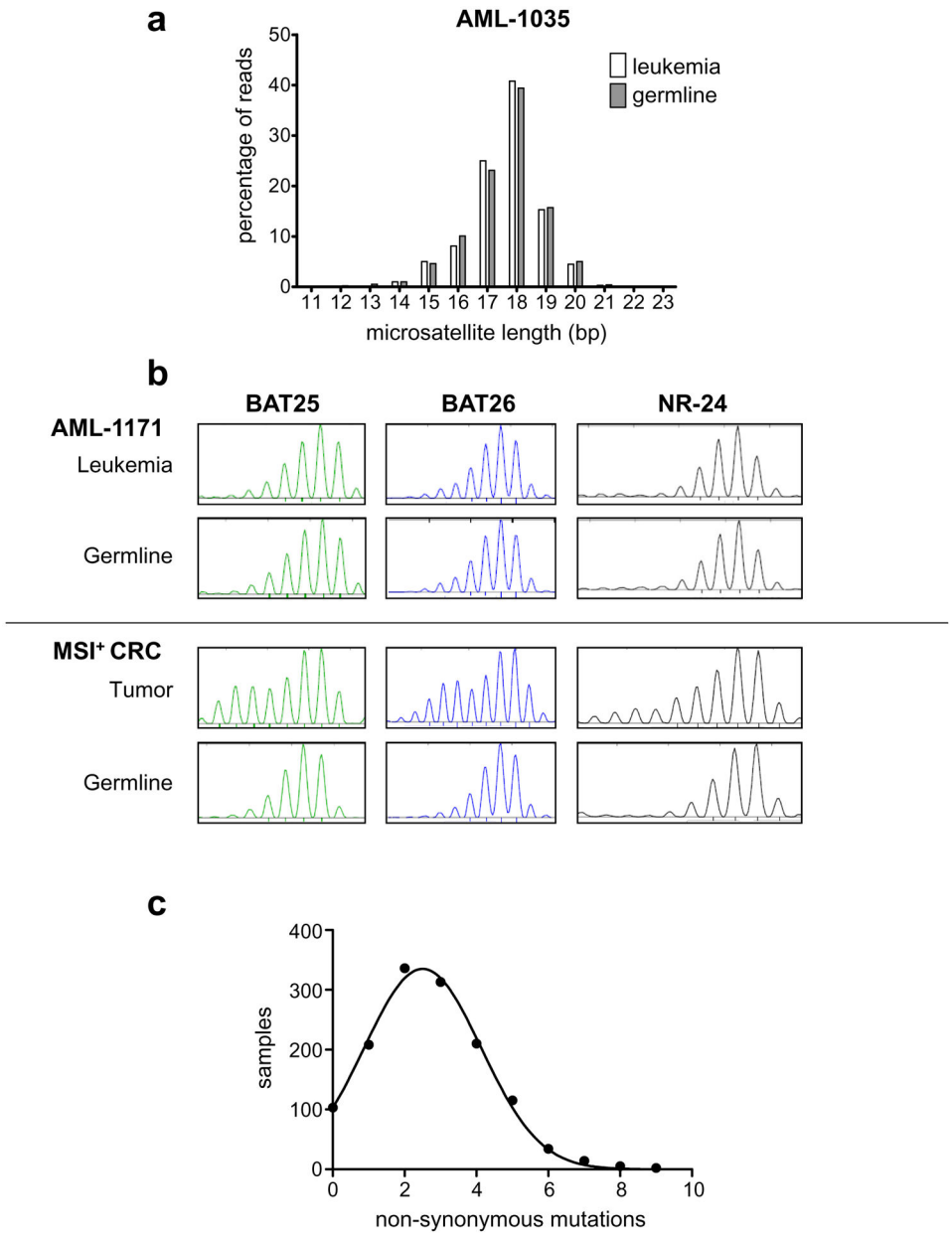

Figure 1.

Microsatellite instability (MSI) detection by next-generation sequencing and capillary electrophoeresis. (**a**) Microsatellite lengths were compared between leukemic and paired germline DNAs by compiling the percentage of reads containing each microsatellite length for each locus. Bar graph shows an example of microsatellite length distribution for a T_{18} mononucleotide run (chr10:112360316-33) in the samples from patient AML-1035. The similar read distributions in both the leukemia and paired germline DNA samples show that this patient does not exhibit MSI. (**b**) Sample traces from conventional PCR/electrophoersis MSI detection examining the indicated microsatellites (BAT25, BAT26 and NR-24) for patient AML-1711. None of the AML samples showed instability by the conventional MSI detection method. One of the three MSI^+ colorectal cancer (CRC) samples is shown as a

positive control. (**c**) Distribution of non-synonymous mutation counts per patient among 1371 AML samples analyzed using the ~250kb target panel. No hyper-mutated outlier samples were detected, consistent with the absence of MSI.

Table 1

The number of cases with elevated allele counts for each microsatellite

Location (hg19)	Gene	Repeat	No. of samples with typical allele counts	No. of samples with elevated allele counts[a]
chr10:112360316	*SMC3*	(T)18	1357	18[b]
chr12:112944241	*PTPN11*	(A)12	1375	0
chr12:112944752	*PTPN11*	(ATC)15	1375	0
chr12:12045438	*ETV6*	(A)14	1375	0
chr16:24230977	*PRKCB*	(TG)24	1374	1
chr16:81955011	*CLL*	(T)15	1372	3[b]
chr18:42644399	*SETBP1*	(A)10	1375	0
chr18:60791877	*BCL2*	(A)10	1372	3
chr2:198267244	*SF3B1*	(T)13	1372	3
chr2:32655874	*BIRC6*	(T)21	1375	0
chr3:12633425	*RAF1*	(A)16	1375	0
chr7:140477005	*BRAF*	(T)12	1375	0
chr8:117864952	*RAD21*	(A)14	1375	0
chr9:2083337	*SMARCA2*	(T)12	1373	2
chr9:86588186	*HNRNPK*	(A)13	1375	0
chr9:86593247	*HNRNPK*	(A)20	1374	1
chrX:123204979	*STAG2*	(T)14	1338	37
chrX:39930434	*BCOR*	(T)13	1374	1

[a] Elevated allele count for each locus was defined as ≥3 standard deviations plus the mean. No sample was identified as microsatellite unstable.

[b] One sample had elevated allele counts for both chr10:112360316 and chr16:81955011.