

Introduction

There are multiple sophisticated DNA repair mechanisms that are dedicated in protecting genomic integrity. A total of 9 genes viz. MSH2-6 (MutS Homolog), MLH1/3 (MutL Homolog), and PMS1-2 (Post Meiotic Segregation) constitute an intact Mismatch Repair (MMR) system that is normally responsible for regulation of recombination, meiotic crossover, diversification of antibody and also for rectification of the misaligned bases/INDELs [1]. Another single step damage reversal system - DRD (Direct Reversal of DNA Damage) specifically acts upon the pyrimidine dimers and alkylated guanine residues. The O6-methylguanine (O6meG) methyltransferase (MGMT) is a suicidal enzyme that removes the methyl adducts from O⁶-Guanine and in the process inactivates itself [2].

Short tandem repeats (STRs) appear at different frequencies in the exonic, intronic, and the regulatory regions of the DNA [3]. Hyper-variability of these repeats in protein coding and non-coding regions is well characterized for solid tumours, but its occurrence in haematologic malignancies remains to be explored.

Here we propose an alternative hypothesis that in certain complex cases of haematologic cancers, CINs alone may not be the prominent cause.

Materials and Methods

•**Procurement of cell lines**- K562 (CML), KG-1 (AML), Molt-4 (T-ALL), and Daudi (Burkitt's lymphoma), HCT116 (CRC), MCF-7 (Breast cancer) – **MMR proficient and deficient**

•**Collection of Control blood samples** from 5 healthy individuals
Ethical Clearance – Ref.No.VIT/TECH/001/Jan28.2017

•**DNA extraction** (HiPura Mammalian Genomic DNA extraction Kit (MB-506))
•**Quantification** using- 0.8% Agarose gel and NanoDrop spectrophotometer

•**Primer selection** - Sequence Specific to Intron, Exon or Regulatory Tandem Repeats

•**Analysis of splice sites** for individual markers

•**Analysis of Tandem Repeat Instability** - PCR and Capillary Electrophoresis based fragment analysis

•**Statistical analysis** - Two sample F-test

•Establishing the **diagnostic and prognostic** relevance

Results

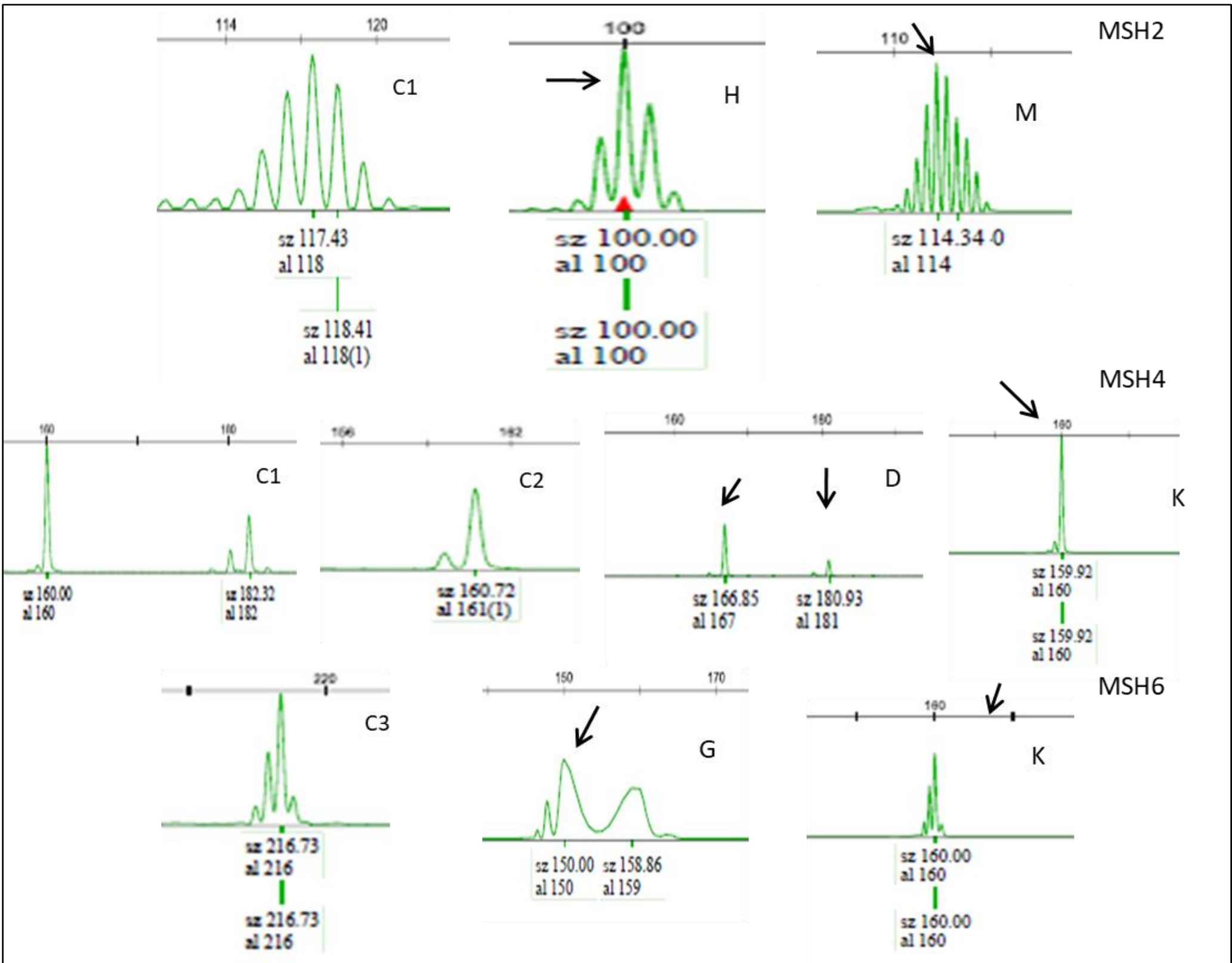


Figure 1: Statistically significant tandem repeat instability in markers of MSH2, MSH4 and MSH6; Arrows indicate shift in the peaks, c1-3- control, H-HCT116, K-K562, G- KG-1, D- Daudi, M- Molt4

Table 1: Percentage distribution of repeat mutability in six cell lines under study

Cell lines	Number of unstable genes	List of unstable genes	Percentage distribution of hypervariability
HCT116	7/10	MSH2/3/4/6, MLH1, PMS1, MGMT	70 %
MCF-7	5/10	MSH3-5, MLH3, PMS2, MGMT	50 %
K562	6/10	MSH3-6, PMS1/2	60 %
Daudi	7/10	MSH2-5, MLH1, PMS1, MGMT	70 %
KG-1	10/10	MSH2-6, MLH1/3, PMS1/2, MGMT	100 %
Molt-4	3/10	MSH2-3, PMS1/2	30 %

References

Pecina-Šlaus, N., Kafka, A., Salamon, I., & Bukovac, A. (2020). DOI: 10.3389/fmolb.2020.00122; Chikan, N. A., Bukhari, S., Shabir, N., Amin, A., Shafi, S., Qadri, R. A., & Patel, T. N. (2015). DOI: 10.1371/journal.pone.0127741; Bhattacharya P., Patel T. N. (2018). DOI: 10.1080/10245332.2017.1354428; Anna, A., & Monika, G. (2018). DOI: 10.1007/s13353-018-0444-7; Vaz-Drago, R., Custódio, N., & Carmo-Fonseca, M. (2017). DOI: 10.1007/s00439-017-1809-4.

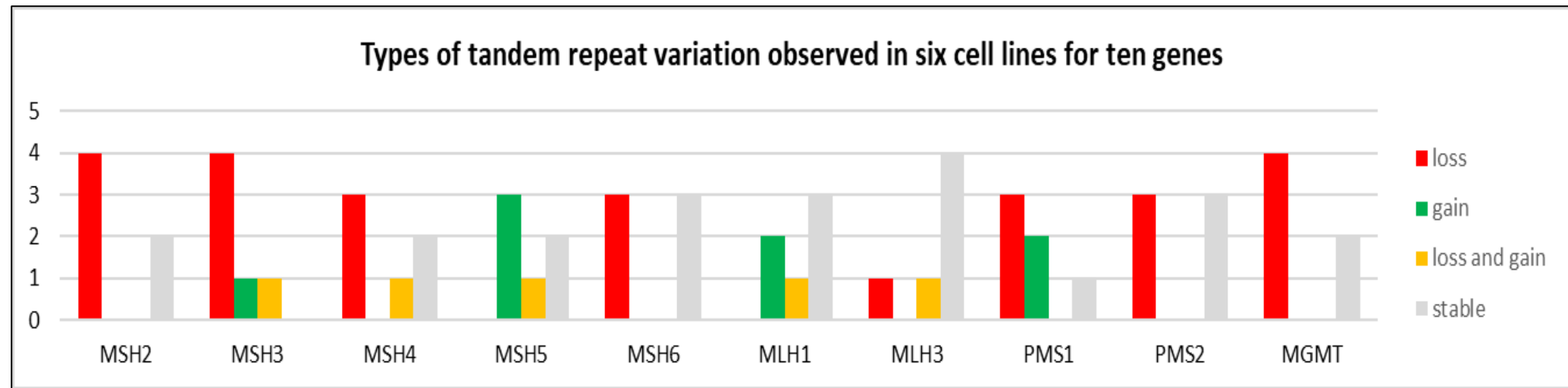


Figure 2: Tandem Repeat Variability as obtained from fragment analysis using the markers for 10 genes in six different cell lines; Red- loss, green- gain, yellow- loss and gain, grey- stable

Discussion

- The markers selected in present study were mostly intronic (MSH3-5, MLH1, PMS1, MGMT) except- MSH2, MLH3 (exon-intron junction), MSH6 (intron-exon junction), and PMS2 (3'-UTR of exon 15).
- The MSH2 (BAT26) and MLH1 (D3S1611) markers were retrieved from peer reviewed literature while others were designed for sequences specific to mononucleotide (MSH5), dinucleotide (MSH3/4, MLH3, PMS1, MGMT) or complex (MSH6, PMS2) repeat regions.
- A statistically significant TR variation was observed for three MMR genes viz., MSH2, MSH4, and MSH6 (P<0.05). KG1 (AML) and Daudi (Burkitt's lymphoma) were found to be highly unstable in terms of repair proficiency.
- The hypermutability in tandem repeats of MSH2 and MLH3 can affect the conserved sequences that define exon-intron boundaries. A prominent sequence contraction at exon-intron junction can lead to misreading of the transcriptional termination and hence introduce/delete sequences in the open reading frame through frameshift.
- The deep intronic repeat variability can impair the recognition and binding of spliceosome at the splice site and activate the cryptic donors and/or acceptors. Altogether it can cause mis-splicing of pre-mRNA, thereby establish an abnormal transcript and novel translated regions [4-5].
- The expansion and deletion in CA-repeat rich regions (D3S1611 of MLH1) can perturb their ability to act as splicing regulatory elements.
- A large deletion in MSH6 intron-exon boundary can eliminate the downstream exon 10 and can result in truncated protein.
- The minimal deregulation (borderline instability- loss of 1bp repeats) at pseudogenic region of PMS2 mandates more research to decipher the extent of their involvement in blood cancer progression.
- In MGMT, the alteration in CA-tract may functionally affect the transferase activity of MGMT, leading to genomic instability.
- Taken together, we suggest that it is high time to accept microsatellite instability in haematologic malignancies. While the MSI profiling and use of Bethesda markers are practiced routinely in diagnostics of solid tumors, the same approach can be undertaken for selective MMR deficient or therapy resistant blood cancer cases, essentially where the cytogenetics fail to assess chromosomal aberrations. Investigating the defective DNA repair (MMR and DRD) in both *de novo* and therapy related cancers can possibly be a potential alternative to deal with selective patients, succumb to morbidity and mortality.

Table 2: Deviation in Number of Repeats as observed for Ten Markers in Six Cell Lines

Cell lines	MSH2	MSH3	MSH4	MSH5	MSH6	MLH1	MLH3	PMS1	PMS2	MGMT
HCT116	~18*	-2 ^{NS}	~1 ^{NS}	0	~55*	~+6/+4*	0	~+18*	0	~8*
MCF-7	0	+19*	0	~+3 ^{NS}	0	0	~1/-4 ^{NS}	0	~1 ^{NS}	~1 ^{NS}
KG-1	~16*	-16*	~1 ^{NS}	~+3 ^{NS}	~66*	~+2 ^{NS}	~+4/-2 ^{NS}	~+6*	~1 ^{NS}	~2 ^{NS}
Daudi	~2 ^{NS}	-18*	~+7/-1	~2/+3 ^{NS}	0	~+6/-2 ^{NS}	0	~13*	0	~2 ^{NS}
Molt-4	~4 ^{NS}	~3/-2 ^{NS}	0	0	0	0	0	~6 ^{NS}	0	0
K562	0	~+6/-2 ^{NS}	~1 ^{NS}	~+3 ^{NS}	~56*	0	0	~2 ^{NS}	~1 ^{NS}	0