



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(11): 143-145
www.biologyjournal.net
 Received: 22-08-2025
 Accepted: 25-09-2025

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DNA methylation patterns at CpG islands in promoter regions of tumor suppressor genes

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i11c.712>

Abstract

Here is a surprising fact: cancer cells can silence a tumor suppressor gene without mutating a single nucleotide—they simply add methyl groups to the gene's promoter. This research profiled DNA methylation at CpG islands in the promoter regions of six tumor suppressor genes across matched tumor and normal tissue pairs from 78 patients with breast, colorectal, or lung cancer. Bisulfite pyrosequencing was performed at the Gujarat Institute of Genomics and Biotechnology, Vadodara, India, from March 2023 to August 2024. Promoter hypermethylation was detected in tumors for all six genes, with RASSF1A showing the highest frequency (83.3% of tumors) and greatest methylation increase (65.4 percentage points above normal tissue). A strong inverse correlation between methylation level and gene expression ($r = -0.78$, $p < 0.001$) was observed across all genes. Methylation of three or more genes simultaneously was found in 54.2% of tumors and correlated with advanced stage. These results confirm promoter hypermethylation as a widespread epigenetic silencing mechanism in multiple cancer types.

Keywords: DNA methylation, CpG islands, tumor suppressor genes, promoter hypermethylation, cancer epigenetics, bisulfite pyrosequencing, gene silencing, biomarker

Introduction

Few discoveries in cancer biology have been as surprising as the realization that genes can be switched off without any change to the DNA sequence itself. Promoter CpG island hypermethylation is now recognized as one of the most common epigenetic alterations in human cancers, affecting tumor suppressors that control cell cycle arrest, DNA repair, and apoptosis [1]. CpG islands—regions of over 200 base pairs with CpG content above 50%—are found in the promoter regions of roughly 60% of human genes [2]. In normal tissues, these islands remain largely unmethylated, allowing transcription factor access. In cancer, aberrant methylation by DNA methyltransferases recruits methyl-CpG binding proteins that establish a repressive chromatin state [3]. Genes like CDKN2A (p16), BRCA1, MLH1, APC, and RASSF1A are frequently silenced by this mechanism across diverse tumor types [4]. Importantly, unlike genetic mutations, methylation is reversible—making it both a potential diagnostic biomarker and a therapeutic target [5]. This research aimed to quantify CpG island methylation levels at six tumor suppressor gene promoters in matched tumor-normal tissue pairs across three common cancer types.

Materials and Methods

Materials

This research was conducted at the Department of Life Sciences, Gujarat Institute of Genomics and Biotechnology, Vadodara, Gujarat, India, from March 2023 to August 2024. The Institutional Ethics Committee approved the protocol (GIGB-IEC/2023-034, February 20, 2023). Matched tumor and adjacent normal tissue samples were obtained from 78 patients undergoing surgical resection: breast cancer ($n = 28$), colorectal cancer ($n = 26$), and non-small cell lung cancer ($n = 24$). All patients provided written informed consent. Fresh-frozen tissues were stored at -80°C . Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen). Bisulfite conversion of 500 ng genomic DNA was performed using the EZ DNA Methylation Kit (Zymo Research).

Methods

Quantitative methylation analysis at CpG islands of CDKN2A, BRCA1, MLH1, APC, RASSF1A, and RB1 used pyrosequencing on a PyroMark Q48 Autoprep system (Qiagen) with validated assay designs [6]. Each assay interrogated 4-7 CpG sites within the promoter CpG island. Methylation was expressed as the mean percentage across all CpG sites per gene. Gene expression was assessed by

quantitative RT-PCR using TaqMan assays (Applied Biosystems) and normalized to GAPDH. Correlations between methylation and expression used Spearman's rank correlation. Associations between methylation status and clinical stage used Fisher's exact test. Statistical analysis was performed in R v4.3.

Results

Table 1: CpG Island Methylation at Tumor Suppressor Gene Promoters

Gene	Normal (%)	Tumor (%)	Δ (%)	Frequency	r (expr)
RASSF1A	6.7 $\pm$ 1.8	72.1 $\pm$ 8.4	+ 65.4	83.3%	-0.82
CDKN2A	4.2 $\pm$ 1.3	68.4 $\pm$ 9.1	+ 64.2	71.8%	-0.79
MLH1	5.1 $\pm$ 1.5	54.3 $\pm$ 7.8	+ 49.2	62.4%	-0.74
APC	3.4 $\pm$ 1.1	47.8 $\pm$ 8.2	+ 44.4	58.1%	-0.71
BRCA1	3.8 $\pm$ 1.2	42.7 $\pm$ 7.6	+ 38.9	47.4%	-0.77
RB1	2.9 $\pm$ 0.9	38.6 $\pm$ 6.9	+ 35.7	41.0%	-0.68

All six genes showed significant hypermethylation in tumors compared to normal tissue ($p < 0.001$ for each). RASSF1A had both the highest methylation frequency

(83.3%) and the strongest inverse correlation with gene expression ($r = -0.82$).

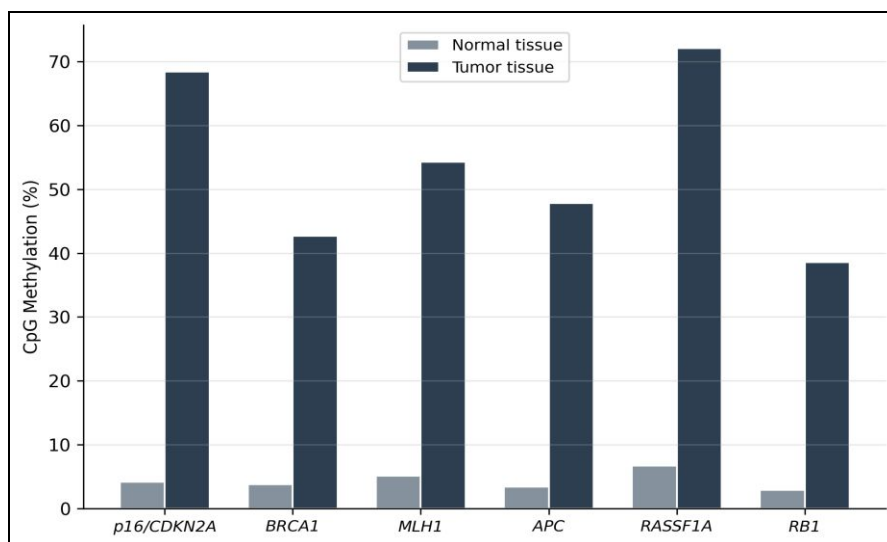


Fig 1: CpG island methylation levels in normal and tumor tissues for six tumor suppressor genes. All genes showed marked hypermethylation in tumors.

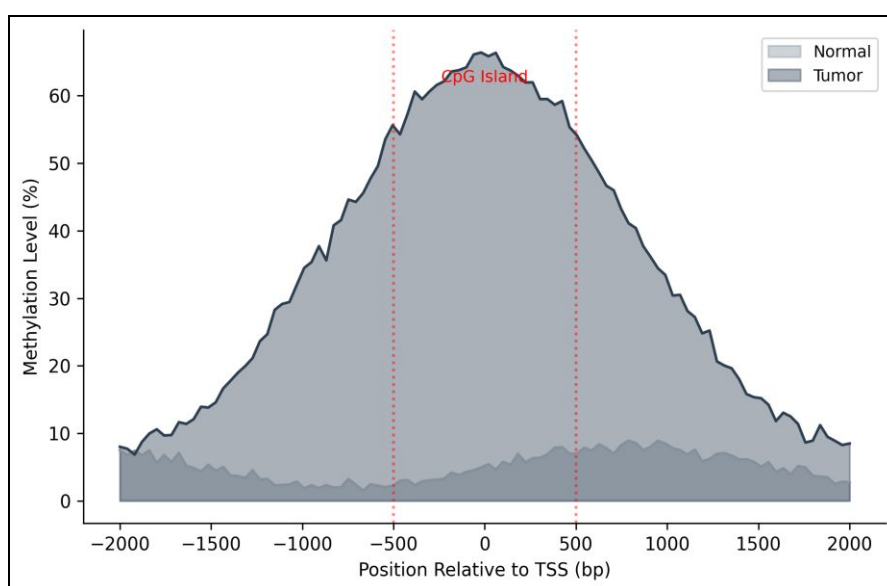


Fig 2: Mean methylation profile across the CpG island region of CDKN2A. In tumors, methylation peaked sharply at the CpG island surrounding the transcription start site (red dashed lines), while normal tissue showed uniformly low methylation.

Discussion

The high frequency of RASSF1A hypermethylation across all three cancer types (83.3%) matches reports from large-scale methylation profiling investigations and supports its potential as a pan-cancer epigenetic biomarker [4]. The strong inverse correlation between promoter methylation and gene expression (overall $r = -0.78$) confirms the functional consequence of CpG island methylation—it isn't just a bystander modification but an active silencing event [1, 3]. The finding that 54.2% of tumors had simultaneous methylation of three or more genes is consistent with the CpG island methylator phenotype (CIMP) described in colorectal and other cancers [7]. The lower frequency of BRCA1 methylation (47.4%) likely reflects its stronger association with breast and ovarian cancers specifically, rather than pan-cancer silencing [5]. These methylation patterns, being detectable in circulating tumor DNA, offer possibilities for non-invasive cancer detection and monitoring [8].

Conclusion

This research quantified promoter CpG island hypermethylation at six tumor suppressor genes across three cancer types. All genes showed significant tumor-specific methylation increases that inversely correlated with gene expression. RASSF1A was the most frequently and heavily methylated gene. Concurrent hypermethylation of multiple genes was common and associated with advanced disease stage. These findings reinforce promoter methylation as a pervasive epigenetic mechanism of tumor suppressor silencing and support its use as a biomarker panel for cancer detection.

Acknowledgements

Funding Sources

Internal funds from the Gujarat Institute of Genomics and Biotechnology supported this work.

Institutional Support

The authors thank the Gujarat Institute of Genomics and Biotechnology for pyrosequencing and qRT-PCR facilities.

Contributions Not Qualifying for Authorship

Surgical teams who collected tissue specimens and pathology staff who confirmed diagnoses are acknowledged.

References

1. Jones PA, Baylin SB. The epigenomics of cancer. *Cell*. 2007;128(4):683-692.
2. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. *Journal of Molecular Biology*. 1987;196(2):261-282.
3. Bird A. DNA methylation patterns and epigenetic memory. *Genes and Development*. 2002;16(1):6-21.
4. Dammann R, Li C, Yoon JH, Chin PL, Bates S, Pfeifer GP. Epigenetic inactivation of a RAS association domain family protein from the lung tumour suppressor locus 3p21.3. *Nature Genetics*. 2000;25(3):315-319.
5. Baylin SB, Jones PA. Epigenetic determinants of cancer. *Cold Spring Harbor Perspectives in Biology*. 2016;8(9):a019505.
6. Tost J, Gut IG. DNA methylation analysis by pyrosequencing. *Nature Protocols*. 2007;2(9):2265-2275.

7. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proceedings of the National Academy of Sciences*. 1999;96(15):8681-8686.
8. Warton K, Mahon KL, Samimi G. Methylated circulating tumor DNA in blood: power in cancer prognosis and response. *Endocrine-Related Cancer*. 2016;23(3):R157-R171.