Methylation Pattern Of MGMT Gene In Relation To Age, Smoking, Drinking And Dietary Habits As Epigenetic Biomarker In Prostate Cancer Patients

Sukhjeet Sidhu1, *Jagdeep S Deep2, R C Sobti3, V L Sharma3, Hitender Thakur3
1Head, Department of Biotechnology, SUSCET, Tangori, Mohali (Pb), India
2Indian Institute of Science Education & Research (IISER), Mohali (Pb), MGSIPA Complex, Sector-26, Chandigarh, India
3Department of Biotechnology, Panjab University, Chandigarh, India

*Correspondence to: Jagdeep S Deep, jagdeep@iisermohali.ac.in, jagdeeps.deep@gmail.com
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Abstract

Epigenetic control of gene transcription is an important step for normal human development and cellular differentiation. Although alterations in DNA methylation pattern is a well-defined epigenetic change linked to human cancers and other diseases, inter-individual epigenetic variations in normal and cancer tissues due to ageing, smoking, drinking and meat-eating are poorly characterized. Population based studies are required to improve our understanding of epigenetic changes leading to progression of cancer. In this study, the methylation pattern of MGMT gene was studied in 100 tissue samples of prostate cancer patients along with 50 tissue samples of Benign Prostatic Hyperplasia cases and 100 blood samples from healthy individuals as controls. The results establish that the methylation pattern increases with age in BPH and healthy individuals whereas the maximum probability of developing prostate cancer is in the age group of 51-60 yrs. It is again proved in the study that smoking, drinking and non-vegetarian diet has a significant contribution in hypermethylation of MGMT gene, thus signifying that the methylation pattern can be designated as epigenetic biomarker in prostate cancer patients where the diagnosis is not well defined in the early stages of tumorigenesis.

Keywords: Methylation; MGMT gene; Epigenetic biomarker.

1. Introduction

Silencing of tumor suppressor and tumor-related genes by hypermethylation at promoter CpG islands [1,2,3,4,5] is one of the major events in human tumorigenesis. The DNA repair protein O6-methylguanine DNA methyltransferase (MGMT) removes alkyl adducts from the O6-position of guanine. MGMT expression is decreased in some tumor tissues, and lack of activity has been observed in some cell lines. Loss of expression is rarely due to deletion, mutation, or rearrangement of the MGMT gene, but methylation of discrete regions of the CpG islands of MGMT [6,7] has been associated with the silencing of the gene in cell lines [4]. In tumorigenesis, the normal functioning of the epigenetic-regulatory system is disrupted leading to inappropriate CpG island hypermethylation and aberrant expression of a variety of genes involved in critical cellular processes. Cancer-dependent epigenetic regulation of genes involved in DNA damage repair, hormone response, cell cycle control, tumor-cell adhesion/metastasis can contribute significantly to tumor initiation, progression, and metastasis, thereby increasing prostate cancer susceptibility, and risk [2]. O6-methylguanine-DNA methyltransferase (MGMT) plays a crucial role in the defence against alkylating agents that generate O6-alkylguanine in DNA, a major trigger of genotoxicity and apoptosis. Therefore, screening individual MGMT expression levels in tumors and normal tissue should predict efficacy of methylation based cancer therapies [8,9,10]. Among the more than 500 primary tumors studied, MGMT hypermethylation was reported in a subset of specific type of cancer [11]. In gliomas and colorectal carcinomas, aberrant methylation has been detected in 40% of the tumors [12], whereas in non-small cell lung carcinomas, lymphomas, and head and neck carcinomas, this alteration has been found in 25% of the tumors [13]. MGMT methylation has not been reported in other carcinomas [14]. This study concluded that epigenetic inactivation of MGMT plays an important role in development of prostate carcinoma. It is the first study to correlate the development of prostate carcinoma with age, smoking, drinking and food habits in North Indian population. We studied the methylation pattern of MGMT gene in 100 tissue samples of prostate cancer patients along with 50 tissue samples of Benign Prostatic Hyperplasia cases and 100 blood samples from healthy individuals as controls. The results signify that the methylation pattern can be designated as epigenetic biomarker [15] in prostate cancer patients.
cancer patients [5], where the diagnosis is not well defined in the early stages of tumorigenesis. Detection of Prostate cancer at early stages could potentially increase survival rates. The purpose of this study is to determine the prevalence of aberrant promoter methylation of MGMT gene [16] in prostate cancer patients [8,9,10]; with a view to designate methylation as prognostic biomarker [17] for prostate cancer patients.

2. Methods

The patients of prostate cancer/BPH were selected from the outpatient departments of PGIMER, Chandigarh, Government Medical College and Hospital, Sector 32, Chandigarh and Government Rajindra Medical College and Hospital, Patiala. Histopathological confirmation of the Prostate cancer was done by Pathologists of the respective institutes. Samples were collected by the clinical staff of these institutes. A detailed questionnaire designed by Indian Council of Medical Research (ICMR), containing all the information about the disease outcome, family history, age, smoking, drinking and food habits was entered at the time of collection of samples. The study proposal and ethics procedure were approved by the ethics review committee of PGIMER, Chandigarh. DNA was extracted from 100 tissue samples of Prostate cancer patients, 50 tissue samples of Benign Prostatic Hyperplasia and 100 blood samples of healthy individuals as controls, using proteinase K digestion followed by standard phenol-chloroform extraction method. In the case of cancer tissue samples, the DNA extraction was done using Sigma GenElute mammalian Genomic DNA Miniprep Kit (G1N70). DNA concentration was measured by running Hind III digested λ marker on 0.8% agarose gel.

2.1 Bisulfite conversion of DNA using Promega Wizard Kit

DNA (2 ug) in a volume of 50 ul was denatured by NaOH (final concentration, 0.2 M) for 10 min at 37°C. 30 microliters of 10 mM hydroquinone (Sigma) and 520 ul of 3 M sodium bisulfite (Sigma) at pH 5, both freshly prepared, were added and mixed, and samples were incubated under mineral oil at 50°C for 16 hr. Modified DNA was purified using the Wizard DNA purification resin according to the manufacturer’s (Promega) prescribed protocol and was eluted into 50 ul of water. Modification was completed by NaOH (final concentration, 0.3 M) treatment for 5 min at room temperature, and was followed by ethanol precipitation. DNA was resuspended in water and was stored at -20°C.

2.2 Bisulfite conversion of DNA using Sigma Kit (Imprint DNA Modification Kit - MOD50-1KT)

DNA samples were modified and then eluted using the prescribed protocol by the manufacturer.

2.3 Methylation specific PCR (MSP)

Methylation specific PCR [18] for MGMT gene using specific primers, Methylated with 98 bp and methylation in relation to food Unmethylated with 108 bp) was done. Taq polymerase was added after 5 minutes. (Hot start) and the programme was run for 95°C-45 seconds, 58°C for 45 seconds and 72°C for 45 seconds. PCR was run for 35 cycles. After keeping the PCR products at 4°C for 1 hour, the methylated as well as unmethylated product was run on 3% Agarose Gel. After successful run, the photograph was taken in the Gel Doc.

3. Results and Discussion

In the present study the methylation pattern of MGMT gene was studied in 100 tissue samples of prostate cancer patients along with 50 tissue samples of Benign Prostatic Hyperplasia cases and 100 blood samples from healthy individuals as controls performing methylation specific PCR (MSP).

3.1 Quality and quantity of genomic DNA

DNA extracted from tissues of Prostate cancer and BPH patients and from blood of healthy controls was checked for their quantity and concentration in solution by electrophoresis on an ethidium bromide stained 1% agarose gel. The sample DNA showed presence of good quality high molecular weight DNA, which was evident from the presence of a single intact band without smearing or degradation. The comparison of band intensities of DNA from blood of lung cancer patients to that of standard 1ug Hind digested lambda DNA molecular weight marker showed almost similar concentrations of DNA in different samples.

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3.2 Primers

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
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<tbody>
<tr>
<td>M: TTTCGAGTTCGTTAGGTTCGC</td>
<td>M: GCACCTTTGAAAGCAAGACG</td>
</tr>
<tr>
<td>U: 5' -TTTGTGTGGATGGTAGTTTTTGT</td>
<td>U: 5' -AACCTGACACTCTTCCCCAAAACAAAAGA</td>
</tr>
</tbody>
</table>

Hypermethylation of the MGMT promoter leads to the loss of its function in various carcinomas, Soejima et al. [19] however, prostate cancer data are not clear. Indeed, some studies have reported a lack of significant MGMT methylation in prostate tumors, Maruyama et al. [20]; Yamanaka et al. [21]; Yegnasubramanian et al. [22] whereas others have detected moderate to high levels, 75.7%; 22%, Kang et al. [23]; Konishi et al. [24].

In the present study, the overall frequency of methylation was found out to be 53%. The frequency of methylation was maximum in subjects in the age group of 41-50 years, declining in a systematic manner with the increase in age i.e. 60% in 51-60 yrs; 58% in 61-70; 52% in 71-80 and touching the lowest percentile of 30 in age group of 81-90 yrs. In case of smokers, the percentage of methylation was 73% as compared to 30% in non-smokers. In the subjects who consumed alcohol, the methylation was 58% as compared to 22% in non-drinkers. In relation to food habits, non-vegetarians showed methylation in 60% of the cases as compared to 20% in vegetarians.

In case of BPH patients, the age group of 61-70 showed maximum methylation of 22% whereas it was 20% in the age group of 51-60 years, whereas methylation was nil in the age groups of 41-50 yrs and 71-80 yrs. In smokers, methylation was observed in 37% of the cases as compared to just 5% in non-smokers. Methylation was observed in 16% of BPH patients who consumed alcohol as compared to nil in non-drinkers. In case of food habits, non-vegetarians showed methylation in 18% of the cases as compared to 10% in vegetarians.

In healthy controls, the frequency of methylation was zero in the age group of 31-40 years and showed a steady increase in methylation with age. It was 22% in age group of 41-50, 36% in age group of 51-60 years, 39% in the age group of 61-70 years and frequency of methylation was 42% in the age group of 71-80 years. Frequency of methylation was found to be more in smokers i.e. 42% as compared to 17% in non-smokers. Drinkers showed frequency of 63% as compared to 18% in non-drinkers. The frequency of methylation was much more in non-vegetarians (49%) than in vegetarians (17%).
Figure 2. Gel illustrating methylation-specific PCR in prostate cancer cases.
U = Unmethylated; M = Methylated; L = Ladder

Figure 3. Gel illustrating methylation-specific PCR in BPH cases.
U = Unmethylated; M = Methylated; L = Ladder

Figure 4. MGMT methylation (cancer patients) in different age groups.
Figure 5. Methylation in MGMT gene (cancer patients) in smokers and non-smokers.

Figure 6. Methylation in MGMT gene (cancer patients) in drinkers and non-drinkers.

Figure 7. Methylation in MGMT gene (cancer patients) in relation to food habits.
Figure 8. Methylation in MGMT gene (BPH) in relation to different age groups.

Figure 9. Methylation in MGMT gene (BPH) in smokers/non-smokers.

Figure 10. Methylation in MGMT gene (BPH) in drinkers/non-drinkers.

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Figure 11. Methylation in MGMT gene (BPH) in relation to food habits.

Figure 12. Methylation in MGMT gene (control) in different age groups.

Figure 13. Methylation in MGMT gene (control) in smokers/non-smokers.
4. Conclusion

It is evident from the study that the methylation increases with age in BPH and Control samples whereas in the cases of cancer patients, the maximum probability of prostate cancer is in the age group of 51-60 and it continues to decrease in a systematic manner with the increase in age. In cases, control and BPH patients the methylation incidence is significantly high in smokers [25], drinkers and meat eaters as compared to non-smokers, non-drinkers and vegetarians. Therefore, we conclude that smoking, consumption of alcohol and non-vegetarian diet can trigger the onset of hypermethylation, which can result in development of carcinoma of prostate. This study clearly demonstrates that hypermethylation of MGMT gene can be designated as epigenetic biomarker [26,27,28] for prognosis of prostate cancer. Although this a first study of its kind in the North Indian population, but the results are in conformity of earlier records in other cancers in other parts of the world [29,30,31].

5. List of Abbreviations

BPH: Benign prostate hyperplasia; MSP: Methylation specific PCR; PCR: Polymerase chain reaction.

6. Competing interests

The authors declare that they have no competing interests.

7. Authors' Contributions

SS and JSD have done the major work, which is a part of PhD project of SS. RCS and VLS both, supervised the PhD project. The manuscript was written and checked by JSD. HT helped in the conduct of experiments.
8. Acknowledgement

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