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Research Paper

An Investigation of DNA Methylation and Roles of The Histone Methyltransferases and Methyl-CpG Binding Protein in Human Cancer Through Gene Expression Profiling

BIOTECHNOLOGY

KEYWORDS : Epigenetics, DNA Methylation, Human Cancer, Histone methyltransferase, UHRF1, MeCP2, EHMT and EZH

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ABSTRACT

Background- A growing number of human diseases has been found to be associated with aberrant DNA methylation and correlates with chromatin associated gene silencing. The recruitment of MeCP2 to methylated CpG dinucleotides represents a major mechanism by which DNA methylation can repress transcription. UHRF1 promotes widespread DNA hypomethylation, an epigenetic hallmark of cancer cells. Histone methyltransferases (HMT) catalyze the transfer of methyl groups to Histone proteins.
Methodology- Standard & Real Time PCR, SDS-PAGE & Western Blotting
Result- The Ct value of genes UHRF1, MeCP2, EHMT1, EHMT2 and EZH2 is slightly higher in Cancerous cells than in normal cells respectively except Ct value of EZH1 is slightly lower in Cancerous cells than in normal cells.
Conclusion- From both of the qualitative analysis and the expression level of all these genes analysed viz. MeCP2, UHRF1, EHMT1, EHMT2, EZH1 and EZH2, slightly indicates overexpression of these genes in Cancerous cells than the Normal cells.

INTRODUCTION

Epigenetic change is a regular and natural occurrence but can also be influenced by several factors including age, environment and disease state. Epigenetic change can have more damaging effects that can result in the development of some cancers. The genome of the transformed cell undergoes simultaneously a global genomic hypomethylation and a dense hypermethylation of the CpG islands associated with gene regulatory regions. At least three systems including DNA methylation, Histone modification and non-coding RNA associated gene silencing are currently considered to initiate and sustain epigenetic change. Typically, there is hypermethylation of tumor suppressor genes and hypomethylation of oncogenes. Methylated CpGs attract methyl-CpG-binding domain proteins that recruit repressor complexes, resulting in Histone modification. Human genes encoding proteins with Histone methyltransferase activity include: EHMT1, EHMT2, EZH1 and EZH2.

UHRF1 (Ubiquitin-Like with PHD and Ring Finger Domains 1) is a Protein Coding gene. Among its related pathways are Chromatin Regulation / Acetylation. Annotations related to this gene include sequence-specific DNA binding transcription factor activity and ubiquitin-protein transferase activity. UHRF1 is a multi-domain protein associated with cellular proliferation and epigenetic regulation. The UHRF1 binds to methylated CpG dinucleotides and recruits transcriptional repressors DNA methyltransferase 1 (DNMT1) and Histone deacetylase 1 (HDAC1) through its distinct domains. The MeCP2 protein contains a methyl CpG binding domain protein in its N-terminus and a TRD (Transcription Repression Domain) in its C terminus. Using a ChIP approach, it was reported that MeCP2 is not always associated with transcriptional repression as it is associated with transcriptionally active genes also. Numerous lysine and arginine residues in the N-terminal tails of Histone H3 and H4 are subjected to methylation by Histone methyltransferase enzymes (HMTs). All of these residues can have mono, di- or tri-methyl groups attached to them. Euchromatic Histone-lysine N-methyltransferase 1 (EHMT1) is an important subunit of H3K9 methyltransferases in humans, encoded by the EHMT1 gene. The protein encoded by this gene is a histone methyltransferase that is part of the E2F6 complex, which represses transcription. The encoded protein methylates the Lys-9 position of histone H3, which tags it for transcriptional repression. This protein may be involved in the silencing of MYC- and E2F-responsive genes and therefore could play a role in the G0/G1 cell cycle transition. The highly similar euchromatic H3K9 methyltransferases EHMT1 and EHMT2 (also referred to as GLP and G9a, respectively) form a heteromeric complex and the loss of either one significantly reduces mono- and dimethylation of H3K9, a marker of silent euchromatin, which is a procedure crucial for the transcription, signal transduction, proliferation and differentiation of cells. Histone methyltransferase specifically mono- and di- methylates 'Lys-9' of Histone H3 (H3K9me1 and H3K9me2, respectively) in euchromatin. H3K9me represents a specific tag for epigenetic transcriptional repression by recruiting HP1 proteins to methylated Histones.

EZH1 (Enhancer of Zeste Homolog 1) is a component of a non-canonical Polycomb Repressive Complex-2 (PRC2) that mediates methylation of Histone H3 lys27 (H3K27) and functions in the maintenance of embryonic stem cell pluripotency and plasticity. EZH2 gene encodes a member of the Polycomb-group (PcG) family. PcG family members form multimeric protein complexes, which are involved in maintaining the transcriptional repressive state of genes over successive cell generations. This protein associates with the embryonic ectoderm development protein, the VAV1 oncoprotein, and the X-linked nuclear protein. This protein may play a role in the hematopoietic and central nervous systems. It has been reported that increased expression of EZH2 had been associated previously with invasive growth and aggressive clinical behaviour in prostate and breast cancer. Amounts of both EZH2 mRNA and EZH2 protein were increased in metastatic prostate cancer.

TECHNIQUES USED

1. Gel Electrophoresis
2. Spectrophotometer
3. Primer Designing
4. Standard PCR
5. Real Time PCR
6. SDS-PAGE
7. Western Blotting

MATERIALS AND METHODS

1. Extraction of total RNA from blood
2. Qualitative Estimation of RNA Concentration by Denaturing Gel Electrophoresis
3. Quantitative Estimation of RNA Concentration by Spectrophotometric Analysis
4. Primer Designing for Standard PCR by PerlPrimer Software
5. Primer designing for Real Time PCR by PerlPrimer Software
6. First strand cDNA synthesis
7. Agarose Gel Electrophoresis of the PCR products
8. Analysis of the Relative Expression level of the different genes
9. Real Time PCR Analysis
10. Total Protein Estimation by Lowry's Method
11. SDS-PAGE & Western Blotting Protocols

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RESULT & DISCUSSION

A total of 4 Cancer patients and 4 normal patients were analyzed taking their blood samples.

Table 1: Real Time PCR Quantitative Result for UHRF1 and MeCP2

Sample	Ct- value of β-actin	Mean	Ct- value of Gene	Mean
S-1 UHRF1	19.58 18.17 18.16	18.63	18.16 17.32 16.12	17.20
S-2 UHRF1	22.12 24.39 21.58	22.69	21.45 23.14 20.22	21.60
S-3 MeCP2	22.86 23.38 21.35	22.53	21.12 21.12 20.23	20.82
S-4 MeCP2	24.36 21.14 20.11	21.87	22.25 20.25 20.02	20.84
S-5 (EHMT1)	18.52 17.12 18.13	17.92	17.45 16.52 17.95	17.30
S-6 (EHMT1)	26.35 23.85 20.86	23.60	24.15 23.14 22.23	23.17
S-7 (EHMT2)	23.46 25.26 26.32	25.68	20.39 21.36 22.45	21.40
S-8 (EHMT2)	25.36 25.14 22.52	24.34	25.25 24.25 21.33	23.61
S-9 (EZH1)	25.22 22.25 25.15	24.20	23.52 21.53 24.22	23.09
S-10 (EZH1)	25.52 22.26 22.27	23.35	23.39 21.23 21.52	22.04
S-11 (EZH2)	24.36 25.33 26.32	25.33	21.12 22.12 25.23	16.15
S-12 (EZH2)	25.36 25.14 23.11	24.53	25.25 24.25 21.33	23.61

Normal Samples: S-1, 3, 5, 7, 9 & 11

Cancerous Samples: S-2, 4, 6, 8, 10 & 12

Electrophoresis detection of total RNA in 1% Agarose gel:-

A B C D E F G H

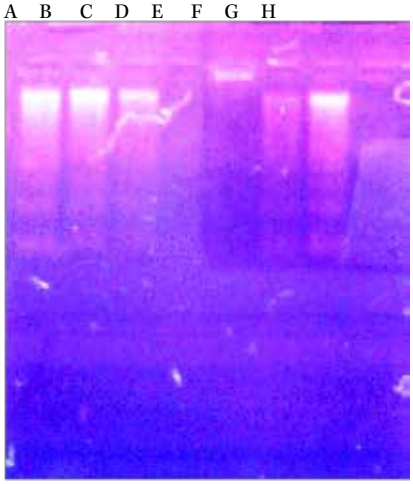

Fig 1: From Normal blood

I J K L M N O

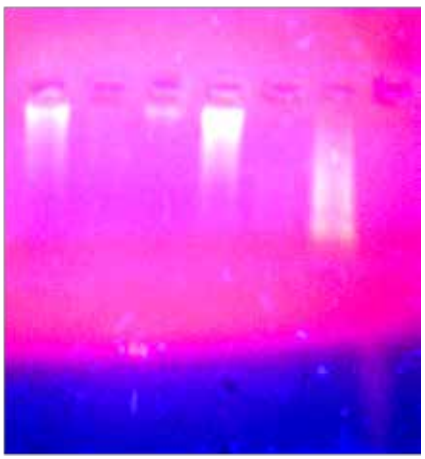

Fig 2: From Cancerous blood

Protein Estimation by Lowry Method

x= protein concentration

x(C1)= 148.33µg/ml, x(C2)= 153.60µg/ml,
x(C3)= 210.61µg/ml, x(N1)= 150.09 µg/ml,
x(N2)= 135.18 µg/ml, x(N3)= 72.02 µg/ml
(Normal- N 1, 2, 3 & Cancer- C 1, 2, 3)

While comparing qualitative analysis of the expression level of Methyl Binding proteins – MeCP2 and UHRF1 and Histone Modifying Enzymes –EHMT1, EHMT2, EZH1, EZH2 in normal and cancerous cells through real time PCR, the Ct value of UHRF1, MeCP2, EHMT1, EHMT2, and EZH2 is slightly lower than the Ct value of **β-Actin or showing down expression hence they are positive with standard value.** The Ct value of genes UHRF1, MeCP2, EHMT1, EHMT2 and EZH2 is slightly higher in Cancerous cells than in normal cells respectively except Ct value of EZH1 is slightly lower in Cancerous cells than in normal cells. While comparing the expression level of the genes EHMT1, EHMT2, EZH1 and EZH2 through SDS PAGE & Western Blotting, no difference was found between normal and cancerous blood cells with protein bands observation. For some reasons, antibodies used against the enzymes (expressed by these genes) may not have been bound. Through Protein Estimation by Lowry Method, it is found that the total protein concentration of cancerous blood cells is slightly higher than the normal blood cells.

From both of the qualitative analysis and the expression level of all these genes analysed viz. MeCP2, UHRF1, EHMT1, EHMT2, EZH1 and EZH2, slightly indicates overexpression of these genes in Cancerous cells than the Normal cells. More Cancerous blood cells should be analysed to conclude if there is significant increase in the expression of these genes.

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