Stability of DNA Methylation of Blood Evidence Under Temperature and Humidity Conditions

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Received 31-10-2021, Accepted 26-10-2021, published 31-05-2022.
DOI: 10.52113/2/09.01.2022/59-69

Abstract: : The stability of deoxyribonucleic acid (DNA) depends on several factors including temperature, humidity, and period conditions. Temperature plays an essential role in DNA degradation, the high relative humidity cause rapid decay of DNA. The aim of this study is to assess the effects of different conditions [Temperature (0°C and 55°C) and Humidity (58% and 12%)] on DNA methylation by bisulfite. The study has investigated 30 samples of blood that have been collected from healthy people. The samples were distributed for four groups each group has 15 samples were exposed at different conditions temperature (0°C, and 55°C) and humidity (58%, and 12%) in different time (0,1,2,4 and 8 days). The DNA methylation of TRIM 59 gene was stable in room temperature whereas in the 0°C and 55°C the results showed that a slight regression in TRIM59gene.

Keywords: TRIM59, DNA methylation, Blood, Pyrosequencing, Temperature, Humidity

Introduction

Covalent addition of a methyl group to a 5´ _ position in the cytosine pyrimidine ring in the DNA sequence is called DNA methylation. A family of enzymes called DNA methyltransferases (DNMTs) catalyzes DNA methylation [1,2] by using S-adenosylmethionine as the methyl group donor [3]. TRIM59 is a novel TRIM family member which is characterized by the presence of a Really Interesting New Gene (RING) finger

domain, a B-box 2domain, two coiled-coil domains and a trans membrane domain in its structure [4]. It is implicated in a wide range of biological processes in lung cancer and other multiple tumors. It may be used as a novel multiple tumor biomarker in immunohistochemically detection for early tumorgenesis [5]. Upregulation of the TRIM59 gene promotes gastric carcinogenesis by facilitating the p53 ubiquitination and degradation [6].

Materials and Methods

Temperature and humidity measurements: The samples were placed in filter papers and incubated in temperature and humidity incubator as illustrated in Table (1). The effects of temperature and humidity were monitored on a day by day basis.

Table 1 Different ranges of temperature and humidity during different periods of the days.

Periods of time	Temperature C°	Humidity %
0	0	58
	55	12
1	0	58
	55	12
2	0	58
	55	12
4	0	58
	55	12
8	0	58
	55	12

DNA Extraction: Peripheral blood samples were collected from 30 healthy individuals from the Iraqi population between 19-45 years of age. Then, blood DNA sample (20µl solution from mixture of spot with reagent (free nuclease water)) placed into a 200µl PCR tube was immediately extracted by

using Quick Blood Genomic DNA Extraction Kit (DSBIO, china).

Bisulfate Sodium Conversion of genomic DNA: For DNA methylation, 500 ng DNA was sodium bisulfate treated using the Methyl Edge Bisulfite Conversion kit according to the manufacturer's protocol (Promega, USA) and then, eluted to the final volume of 46 µL.

PCR: The amplification assay requires two independent primer sets for PCR amplification, one primer labeled by biotin designed to recognize the methylated templates and other primer is to the unmethylated versions of the bisulfitemodified sequence. PCR primers (ADS5738FP and ADS5738RPB) for TRIM59 gene were designed from EpigenDx Company. The CpG loci location or coordinates are based on Ensemble Gene ID (ADS5738-FS2), Ensemble transcript ID (ENST00000309784), and GRCh38 genomic build. PCRs were performed using 1 µL of bisulfite treated DNA, 0.75 U of Qiagen Hot Star Taq Polymerase and 0.6 µM of each primer (ADS9042FPB and ADS9042RP, EpigenDx, USA), 3 µL of 1X PCR buffer, 200 µM of dNTPs and adjusted to a final volume 30 µL. Thermal cycling conditions included a 15min at 95°C initial denaturation followed by 45 cycles of the 30s at 95◦C, 30s at 56◦C, and 30s at 68◦C, ending in a 5min 68◦C final extension and 4◦C ∞.

Pyrosequencing: Pyrosequencing is performed using PSQ 96HS system or PSQ 96HSA system for 30 blood samples were exposed in different conditions including temperature at 0°C, humidity (RH) at 58%, at 55°C, at RH 12%, and at five periods of days (0, 1, 2, 4, and 8). The systems are equivalent to

the Pyro Mark MD system. The run of pyrosequencing as the manufacturer's instruction.

Results and Discussion

TRIM59 gene contains four sites that are CpG1, CpG2, CpG3 and CpG4. From the results shown in table (2 and 5), it appeared that all 4 CpG sites belonging to TRIM59 gene have significant linear relationship in the different conditions (temperature and humidity) (all P-values<0.05, 0.01). The fitting process using the simple regression model that illustrated in Table (3) and (Fig. 1) provided good correction coefficients. In this study, the assay of Human tripartite motif containing 59gene located at Chromosome Chr3:160450365-160450349 . This assay was designed to target Promoter ADS5738-FS2 Ensemble Gene ID ENSG00000213186 at distance transcription start site (TSS) -536 to -520. TRIM59 gene showed four CpG sites detected at which are: CpG1, Chr3:160450349 CpG2, Chr3:160450355 CpG3, Chr3:160450362, CpG4, Chr3:160450365 Four CpG sites have appeared significant linear relationship with the different conditions (temperature and humidity), (all P-values<0.05, 0.01) and identified the methylation status was stable correlated with the perfect conditions (temperature and humidity). This study compatible with previous study that showed temperature and relative humidity levels associated with methylation , temperature and relative humidity may also interact to produce stronger effects. The DNA methylation in perfect conditions (temperature 25°C) was a stable [7]. This study compatible with previous study that showed a strong correlation with age and age associated hyper methylation at TRIM59 CpG

islands [8]. Gene family implicated in a number of critical processes including, proliferation, immunity, transcriptional regulation, antiviral, neuro-development, cancer and cell differentiation. However, the function of most TRIM family members is poorly understood and was supposed only based on computational analysis from their RBCC (RING finger, B-box, Coiled-Coil) domains. The domain of RING (Really Interesting New Gene) is frequently involved in proteolysis acting as the ubiquitin-proteasome system and E3 ubiquitin ligases in the regulation of numerous cellular processes including cell cycle regulatory transcription factors, proteins, and signal transducers. While in the different conditions (0°C and RH 58% and 55°C and RH 12%) and at the different times of days (0,1,2,4, and 8), the results which taken from random DNA blood samples showed the regression between DNA quantity for both (Autosomal and Y) and the CpG of TRIM59 mean shown that a very less regression in some samples than the other samples which have shown slight regression.

Table 2 The relationship between CpG sites of TRIM59 gene and mean at different conditions (temperature 0°C, RH 58%, and at 0,1,2,4,8 days).

Periods of time	CpG 4	CpG 3	CpG 2	CpG 1	Mean
0	5 7.3	6 6.4	60.8	55.4	60.0
	56.5	69.4	63.3	56.9	61.5
	57.6	66.5	60.3	55.7	60.0
1	6 0.4	7 3.2	70.5	67.5	67.9
	4 8.5	5 4.4	49.5	43.5	49.0
	6 9.7	7 9.7	72.7	69.1	72.8
2	6 3.4	6 8.0	61.9	58.2	62.9
	6 3.3	6 8.9	63.0	58.3	63.4
	6 4.1	6 5.9	60.7	55.4	61.5
4	5 3.8	6 1.3	55.5	49.7	55.1
	5 9.0	6 8.9	63.6	60.9	63.1
	5 6.5	6 7.0	60.5	57.9	60.5
8	6 0.9	6 8.8	63.2	58.6	62.9
	6 7.1	72.2	66.7	63.6	67.4
	6 4.9	70.0	66.7	61.9	56.9

Table 3 The relationship between Autosomal (FAM) DNA quantitation and the mean of CpG sites of TRIM59 gene at the 0°C and humidity 58% in the (0,1,2,4, and 8 days).

Periods of time	DNA quantity (FAM)	Mean of CpG
0	0.07	60
	0.16	61.5
	0.09	60
1	0.18	67.9
	0.09	49
	0.41	72.8
2	0	62.9
	0	63.4
	0.02	61.5
4	0	55.1
	0.02	63.1
	0.03	60.5
8	0.07	62.9
	0.004	67.4
	0.08	56.9

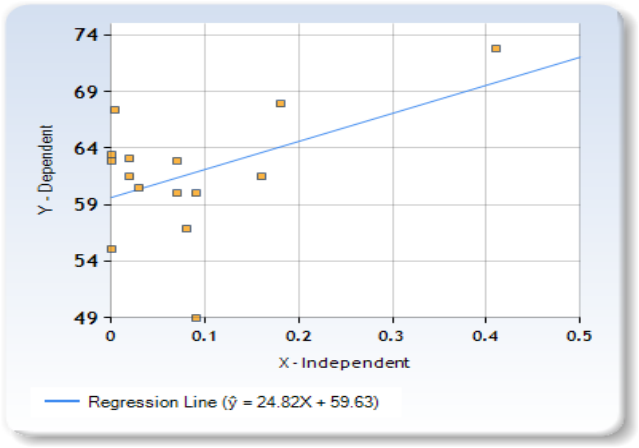

Fig 1 The regression between Autosomal DNA quantitation on the x axis and the mean of CpG sites of TRIM59 gene on the y axis at the 0°C and humidity 58% in the (0,1,2,4, and 8 days).

Table 4 The relationship between (Y) DNA quantitation and the mean of CpG sites of TRIM59 gene at the 0°C and humidity 58% in the (0,1,2,4, and 8 days).

Periods of time	DNA quantity (Y)	Mean of CpG
0	0.13	60
	0.19	61.5
	0.13	60
1	0	67.9
	0	49
	N/A	72.8
2	N/A	62.9
	N/A	63.4
	N/A	61.5
4	0	55.1
	N/A	63.1
	0	60.5
8	0	62.9
	N/A	67.4
	N/A	56.9

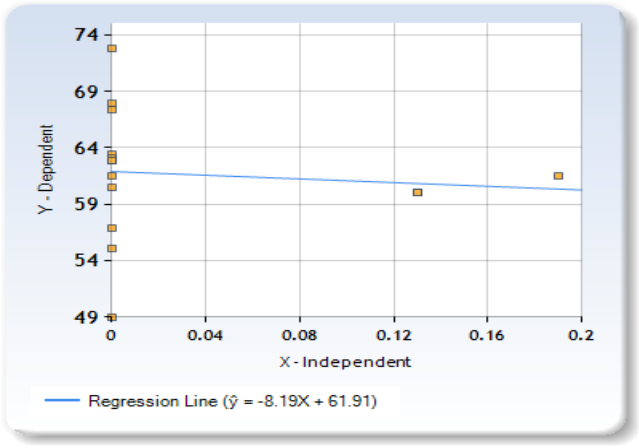

Fig 2 The regression between (Y) DNA quantitation on x axis and the mean of CpG sites of TRIM59 gene on the y axis at the 0°C and humidity 58% in the (0,1,2,4, and 8 days).

Table 5 The relationship between CpG sites of TRIM59 gene and mean at different conditions (temperature 55°C, RH 12%, and at 0,1,2,4,8 days).

Periods of time	CpG4	CpG3	CpG2	CpG1	Mean
0	41.2	51.9	46.0	40.2	44.8
	41.8	53.0	44.3	34.3	43.4
	41.7	46.4	43.8	39.0	42.7
1	43.5	54.9	51.4	41.7	47.9
	48.6	55.8	45.7	42.2	48.1
	46.7	56.3	50.8	43.5	49.3
2	51.2	58.9	52.8	45.7	52.1
	43.5	49.9	43.1	36.6	43.3
	43.2	50.2	45.2	39.4	44.5
4	51.8	54.5	49.8	47.8	51.0
	40.0	47.1	41.7	37.6	41.6
	42.6	53.7	49.2	41.1	46.7
8	44.8	53.1	46.7	43.9	47.1
	44.6	51.7	45.3	39.6	45.3
	47.2	55.3	48.2	49.0	49.9

Table 6 The relationship between Autosomal (FAM) DNA quantitation and the mean of CpG sites of TRIM59 gene at the 55°C and humidity 12% in the (0,1,2,4, and 8 days).

Periods of time	DNA quantity (FAM)	Mean of CpG
0	0.49	44.8
	0.02	43.4
	0.78	42.7
1	0	47.9
	0	48.1
	N/A	49.3
2	0.36	52.1
	0.25	43.3
	0.21	44.5
4	0.34	51
	0.54	41.6
	0.21	46.7
8	0	47.1
	0.38	45.3
	0.41	49.9

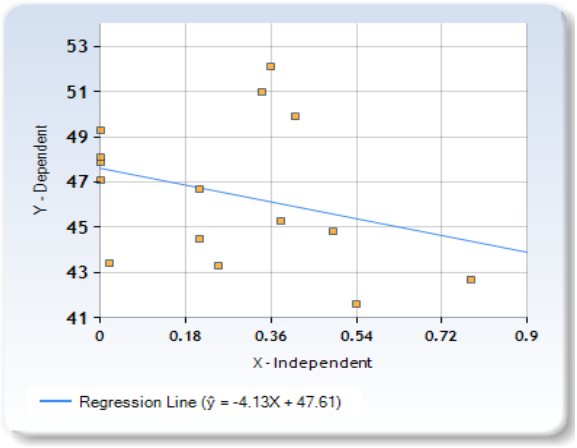

Fig 3 The regression between (Y) DNA quantitation on the x axis and the mean of CpG sites of TRIM59 gene on the y axis at the 55°C and humidity 12% in the (0,1,2,4, and 8 days).

Table 7 The relationship between (Y) DNA quantitation and the mean of CpG sites of TRIM59 gene at the 55°C and humidity 12% in the (0,1,2,4, and 8 days).

Periods of time	DNA quantity (Y)	Mean of CpG
0	N/A	44.8
	0	43.4
	0	42.7
1	N/A	47.9
	N/A	48.1
	N/A	49.3
2	0.18	52.1
	0.04	43.3
	0.08	44.5
4	0.09	51
	0.1	41.6
	0.05	46.7
8	N/A	47.1
	0.04	45.3
	0.35	49.9

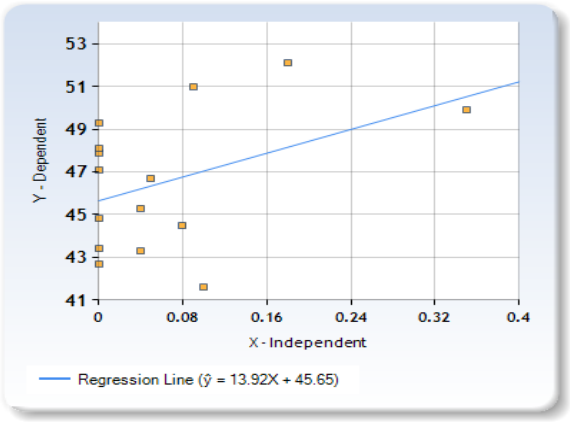

Fig 4 The regression between (Y) DNA quantitation on the x axis and the mean of CpG sites of TRIM59 gene on the y axis at the 55°C and humidity 12% in the (0,1,2,4, and 8 days).

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