**Fusaric acid induced promoter methylation of DNA methyltransferases triggers DNA hypomethylation in human hepatocellular carcinoma (HepG2) cells**

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**Supplementary Information**

**Quantification of DNA methylation formula:**

5-Methylcytosine (ng) =

5-Methylcytosine (%) =

**Quantification of promoter methylation formula:**

Methylation (%) = 100 X 2-ΔCt, where ΔCt = Ct (test) – Ct (reference)

**Supplementary Table S1: The effect of FA on the mRNA expression of *MBD1*, *MBD2*, *MBD3*, *MBD4*, *MBD5* and *MBD6* in HepG2 cells**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Concentration of Fusaric Acid (µg/ml)** | ***p* value** |
| **0** | **25** | **50** | **104** | **150** |
| ***MBD1*** | 1.02±0.01 | 0.38±0.03\*\*\* | 0.42±0.08\*\*\* | 0.18±0.03\*\*\* | 0.48±0.03\*\*\* | <0.0001 |
| ***MBD2*** | 1.02±0.01 | 0.17±0.01\*\*\* | 0.64±0.08\*\*\* | 0.14±0.01\*\*\* | 0.09±0.01\*\*\* | <0.0001 |
| ***MBD3*** | 1.02±0.01 | 0.40±0.05\*\*\* | 0.31±0.02\*\*\* | 0.34±0.08\*\*\* | 0.34±0.03\*\*\* | <0.0001 |
| ***MBD4*** | 1.02±0.01 | 0.14±0.01\*\*\* | 0.67±0.09\*\*\* | 0.20±0.04\*\*\* | 0.22±0.04\*\*\* | <0.0001 |
| ***MBD5*** | 1.02±0.01 | 0.16±0.11\*\*\* | 0.31±0.13\*\*\* | 0.28±0.04\*\*\* | 0.13±0.07\*\*\* | <0.0001 |
| ***MBD6*** | 1.02±0.01 | 0.18±0.01\*\*\* | 0.15±0.02\*\*\* | 0.18±0.03\*\*\* | 0.72±0.06\*\*\* | <0.0001 |

RNA isolated from control and FA treated HepG2 cells were reverse transcribed into cDNA and analysed for *MBD1*-*MBD6* expression by qPCR. Results are represented as mean relative fold-change ± SD (n=3). **Key:** \*\*\**p* < 0.0001, denotes statistical significance, one-way ANOVA with the Bonferroni multiple comparisons test.

**Supplementary Table S2: qPCR primer sequences and annealing temperatures**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **GenBank Accession no.** | **Sense Primer (5’→3’)** | **Anti-Sense Primer (5’→3’)** | **Annealing Temperature (°C)** |
| ***Promoter Methylation*** |
| ***DNMT1*** | NM\_001130823 | ACCGCTTCTACTTCCTCGAGGCCTA | GTTGCAGTCCTCTGTGAACACTGTGG | 60 |
| ***DNMT3A*** | NM\_175629 | GGGGACGTCCGCAGCGTCACAC | CAGGGTTGGACTCGAGAAATCGC | 58 |
| ***DNMT3B*** | NM\_006892 | CCTGCTGAATTACTCACGCCCC | GTCTGTGTAGTGCACAGGAAAGCC | 58 |
| ***MBD2*** | NM\_003927 | AGGTAGCAATGATGAGACCCTTTTA | TAAGCCAAACAGCAGGGTTCTT | 60 |
| ***miR-29b*** | **-**  | TCCGTATGCTGGTTACTCAC | ATTCTGATAAAACCACCAACT | 54 |
| ***Gene Expression*** |
| ***DNMT1*** | NM\_001130823 | ACCGCTTCTACTTCCTCGAGGCCTA | GTTGCAGTCCTCTGTGAACACTGTGG | 60 |
| ***DNMT3A*** | NM\_175629 | GGGGACGTCCGCAGCGTCACAC | CAGGGTTGGACTCGAGAAATCGC | 58 |
| ***DNMT3B*** | NM\_006892 | CCTGCTGAATTACTCACGCCCC | GTCTGTGTAGTGCACAGGAAAGCC | 58 |
| ***Sp1*** | NM\_138473 | CTTGGTATCATCACAAGCCAGTT | TCCCTGATGATCCACTGGTAGTA | 56 |
| ***UHRF1*** | NM\_001048201 | GCCATACCCTCTTCGACTACG | GCCCCAATTCCGTCTCATCC | 58 |
| ***USP7*** | NM\_003470 | GGAAGCGGGAGATACAGATGA | AAGGACCGACTCACTCAGTCT | 58 |
| ***MBD1*** | NM\_015846 | AAGTCTTTCGCAAGTCAGGGG | TCAGCTCAACTTTGCTTCGGA | 58 |
| ***MBD2*** | NM\_003927 | AGGTAGCAATGATGAGACCCTTTTA | TAAGCCAAACAGCAGGGTTCTT | 60 |
| ***MBD3*** | NM\_001281453 | CAGCCGGTGACCAAGATTACC | CTCCTCAGCAATGTCGAAGG | 58 |
| ***MBD4*** | NM\_003925 | TCTAGTGAGCGCCTAGTCCCAG | TTCCAATTCCATAGCAACATCTTCT | 60 |
| ***MBD5*** | NM\_018328 | GGTCTTCCAGCTATACAAGTTCC | ACCTGCTCCAAGCAAGATAAC | 56 |
| ***MBD6*** | NM\_052897 | GGAGTGTCCACTTAATGTCCCC | GTTGCACAGCTTGGTCATGTC | 58 |



**Supplementary Figure S1:** **CpG islands within the *DNMT1*, *DNMT3A*, *DNMT3B* and *MBD2* promoter regions obtained using the MethPrimer software version 2.0. [1].**

**Reference**

[1] Li LC, Dahiya R. MethPrimer: designing primers for methylation PCRs. Bioinformatics. 2002 Nov;18:1427-1431. PubMed PMID: 12424112.