Proceedings of the Nutrition Society (2018), **77 (OCE4)**, E173 doi:10.1017/S0029665118001799

Summer Meeting, 10–12 July 2018, Getting energy balance right

DNA methylation analysis identifies profiles of cervical cancer cells DNA methylation associated with methyl donor nutrients depletion

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Human papillomavirus (HPV) is the most common cause of cervical cancer. However, not all women who acquire HPV infection develop cervical cancer[1]: about 60 % of HPV infections are transient and resolve on their own due to innate and adaptive immunity[2]. Among other factors known to influence risk of cervical cancer are high parity and use of oral contraceptives, while dietary factors influencing the methyl donor cycle may also be important[3]. Dietary methyl donor nutrients are required for maintenance of DNA methylation, modification of which is associated with cancer progression through dysregulation of gene expression[4]. The aim of the present study was to investigate how the availability of methionine and folate impacts globally and specifically on DNA methylation in a C4-II cervical cancer cell model of methyl donor depletion.

The C4-II cervical cancer cells were grown for 8 days either in complete Dulbecco's Modified Eagle's Medium (medium), methionine-depleted medium, folate-depleted medium, methionine-and-folate depleted medium or 5 % methionine-and-folate replete medium. Experiments were conducted in triplicate. DNA was extracted using the Qiagen Blood and Cell Culture DNA Midi Kit and was subjected to sodium bisulfite modification using the EZ DNA Methylation Kit. Methylation profiling was performed using Infinium Methylation EPIC BeadChip at 866895 CpG sites. A comprehensive analysis of DNA methylation data was conducted using the RnBeads package on the R platform.

C4-II cells grown in depleted medium showed more than 19-fold reduction in intracellular folate and 80 % reduction of methionine concentration (data not shown). More than 10000 CpG sites were differently methylated ($p < 0{\cdot}05$) in C4-II cells grown in methionine-depleted and methionine and folate-depleted medium compared with cells grown in complete medium (Fig. 1a & 1b). Cells grown in medium depleted only of folate showed no significant changes in methylation compared to the control group (Fig. 1a & 1c).

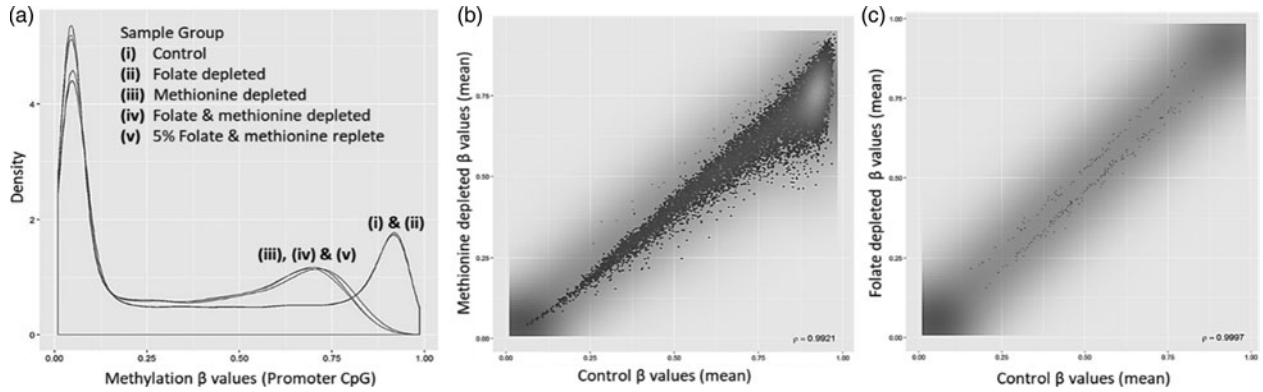

Fig. 1. Distribution of methylation values for all interrogated CpGs from C4-II cells grown in different media conditions (a). Comparison of mean methylation levels between control and folate depleted (b), control and methionine depleted (c) where each plot represents a significant difference between the control and depleted group (p-value <0·05, limma analysis).

Altogether, our data show that depletion of folate has little impact on DNA methylation in C4-II cervical cancer cells, whereas methionine depletion has a significant impact on DNA methylation.

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