

AGE-DEPENDENT DNA HYPOMETHYLATION AS A CONTRIBUTING FACTOR TO CHOLINE DEFICIENCY INDUCED LIVER CANCER DEVELOPMENT

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Dietary choline deficiency causes liver tumor development. Since choline is a major dietary methyl donor we examined the possibility that age-dependent global DNA demethylation may be an epigenetic change that could contribute to hepatocellular carcinoma development. Previous studies have shown that promoter CpG islands in various cancer-related genes are aberrantly methylated and that global DNA is hypomethylated in many cancers. More than 90% of all 5-methylcytosines lies within the CpG islands of the transposons including the long/short interspersed nucleotide element (LINE and SINE). Methylcytosines in LINE-1 is considered to limit the ability of retrotransposons to be activated and transcribed, therefore hypomethylation in LINE-1 could be the root cause of aberrant methylation of other tumor related genes and genome instability. Also, LINE-1 methylation status has been shown to be a good indicator of genome-wide methylation. The methylation status of LINE-1 was assessed by combined bisulfite restriction analysis (COBRA) in which the amount of bisulfite-modified and RsaI-cleaved DNA was quantified using gel electrophoresis. The abundance of unmethylated cytosine in LINE-1 in DNA of CD livers increased as a function of feeding period time, i.e., 11.1% (1 week) to 19.3% (56 weeks), while in control choline-sufficient (CS) livers it increased from 9.2% to 12.9%, indicating that age had a significant influence on LINE-1 methylation and CD-diet had an additional effect. In the 24- and 56-week fed animals, the DNA cytosine content in tumors was higher by approximately 6%. These results clearly indicate that there was genome-wide hypomethylation in CD-livers both in the tumor as well as the non-tumor tissues, in a feeding-period age-dependent manner. This could be the root cause of aberrant methylation in other genes. (*This research was supported in part by NIH R01 CA-82506*)