REVIEW

# Molecular alterations in hepatocarcinogenesis induced by dietary methyl deficiency

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A chronic deficiency of major dietary methyl group donors – methionine, choline, folic acid, and vitamin $B_{12}$ – can induce the development of liver cancer in rodents. Feeding methyl-deficient diets causes several molecular alterations, including altered lipid metabolism, oxidative stress, deregulated one-carbon metabolism, and a number of epigenetic abnormalities that result in progressive liver injury culminating in the development of primary liver tumors. Importantly, this methyl-deficient model of endogenous hepatocarcinogenesis is one of the most relevant models of human liver carcinogenesis that allows studying liver cancer pathogenesis by substantially complementing many shortcomings of humans-only studies. In this review, we describe molecular changes and their role in pathogenesis of liver carcinogenesis induced by methyl deficiency.

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## 1 Introduction

Emerging evidence suggests that nutritional status plays an important role in etiology and pathogenesis of cancer by causing and/or enhancing tumor development, or by preventing and/or inhibiting tumorigenesis [1–5]. This is supported by a wealth of epidemiological evidence showing positive or negative correlations between the intake of nutrients and human cancer incidence [6–8] and is also supported by numerous experimental observations showing that the tumor incidence in various animal cancer models can be profoundly influenced by manipulating dietary components [2]. Uncovering the mechanisms underlying the action of dietary components associated with the oncogenic processes is important for understanding the molecular events that promote tumorigenesis and may play a role in cancer prevention.

A chronic deficiency of the major dietary methyl group donors – methionine, choline, folic acid, and vitamin $B_{12}$ – can induce the development of liver cancer in rodents, as was first reported by Copeland and Salmon in 1946 [9]. Over the years, a number of comprehensive studies have demonstrated that diets lacking methyl donors may act as co-carcinogens [10–12] and, more importantly, as complete carcinogens that can induce liver tumor formation in the absence of any exogenous carcinogens [11, 13–18]. All formulations of ''lipogenic methyl-deficient diets'', i.e. choline-deficient, methionine-choline-deficient, methionine-choline-folic acid-deficient [14–18], provoke several similar molecular alterations, including altered lipid metabolism, oxidative stress, deregulated one-carbon metabolism, and a number of epigenetic abnormalities that result in progressive liver injury culminating in the development of primary liver tumors. More importantly, this methyl-deficient model of endogenous hepatocarcinogenesis is one of the most relevant models to study the pathogenesis of (i) human liver carcinogenesis [17, 19] because the sequence of pathological and molecular events is remarkably similar to the development of human hepatocellular carcinomas; and (ii) non-alcoholic fatty liver disease/non-alcoholic steatohepatitis

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**Abbreviations: RNS,** reactive nitrogen species; **ROS,** reactive oxygen species; **SAH,** *S*-adenosyl-L-homocysteine; **SAM,** *S*-adenosyl-L-methionine

 

[20, 21], a major risk factor for development of hepatocellular carcinomas in developed countries [22, 23].

In this review, we describe molecular changes induced by methyl-deficient diets and highlight their role in pathogenesis of liver carcinogenesis.

## 2 Deregulated one-carbon metabolism

Methionine, choline, folic acid, and vitamin $B_{12}$ are essential for the proper functioning of several main interdependent cellular metabolic processes critical for the biosynthesis of *S*-adenosyl-L-methionine (SAM), the major methyl donor for cellular methylation reactions. SAM is synthesized from L-methionine and adenosine by an ATP-dependent reaction catalyzed by methionine adenosyltransferase [24] in the cytosol of every cell (Fig. 1); however, the liver is the primary location for SAM biosynthesis and degradation [25]. SAM is a key component in transmethylation, transsulfuration, and polyamine synthesis pathways [26, 27]. In the transmethylation pathway, SAM donates its methyl group to a broad spectrum of substrates, i.e. phospholipids, proteins, neurotransmitters, DNA, and RNA, in reactions catalyzed by substrate-specific methyltransferases. In the transsulfuration pathway, SAM is an important precursor for glutathione biosynthesis. In the synthesis of polyamines, SAM is decarboxylated and the remaining propylamino moiety is donated to either putrescine to form spermidine and methylthioadenosine or to spermidine to form spermine and methylthioadenosine.

In the transmethylation pathway, after transfer of the methyl group, SAM is converted to *S*-adenosyl-L-homocysteine (SAH) within the active site of the methyltransferase enzyme [28]. SAH is a potent competitive inhibitor of virtually all methylation reactions [26, 29], and since most of cellular methyltransferases bind SAH with greater affinity than SAM, the efficiency of methyltransferase reactions is absolutely dependent upon the prompt removal of SAH. This is effectively achieved by SAH hydrolase, which

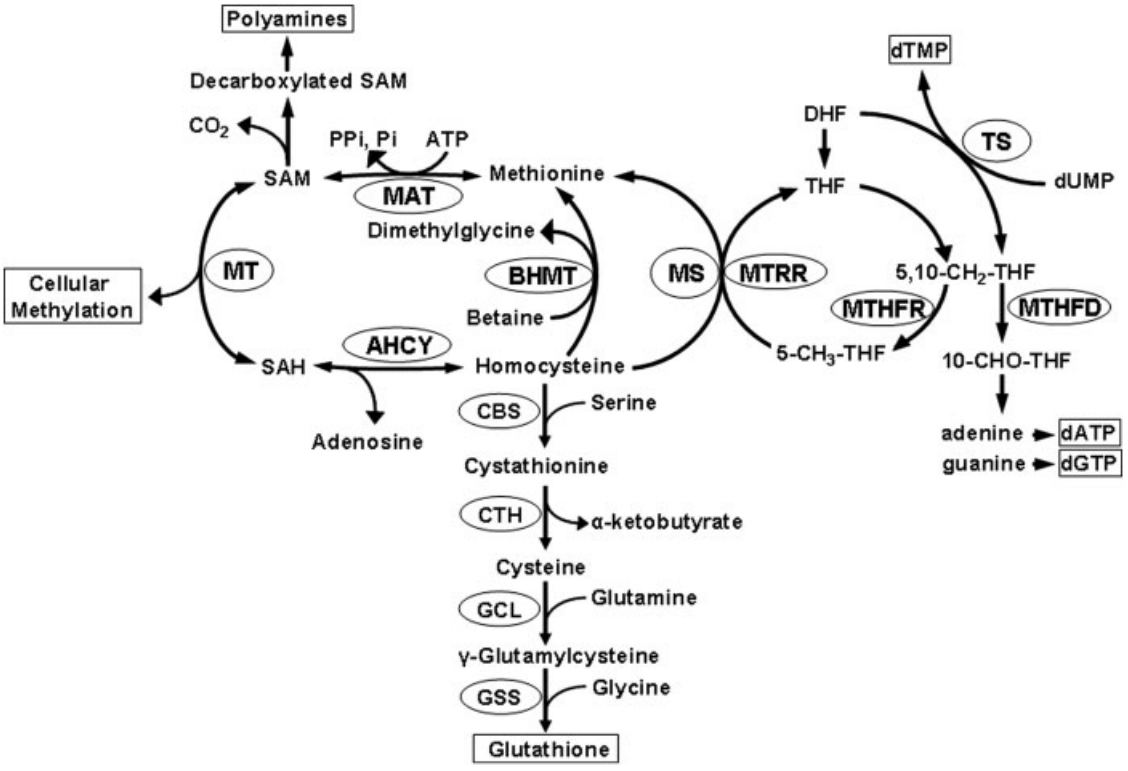


**Figure 1.** Pathways of methionine metabolism in mammalian cells. *S*-Adenosylmethionine (SAM) is synthesized from L-methionine and adenosine by an ATP-dependent reaction catalyzed by methionine adenosyltransferase (MAT). In the transmethylation pathway, SAM donates its methyl group in cellular methylation reactions catalyzed by methyltransferases (MT) and is converted to *S*-adenosylhomocysteine (SAH). In polyamine synthesis, SAM is decarboxylated and the remaining propylamino moiety is donated for biosynthesis of polyamines. SAH is hydrolyzed by SAH hydrolase (AHCY) to homocysteine and adenosine. Homocysteine can be (i) remethylated to methionine either in a folate/$B_{12}$-dependent methionine synthase (MS) reaction or a betaine-homocysteine methyltransferase (BHMT) reaction; or (ii) converted to glutathione by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CTH) in a transsulfuration pathway. Additionally, these metabolic pathways are tightly connected with folate-dependent DNA synthesis, since 5,10-methylenetetrahydrofolate (5,10-$CH_2$-THF) is not only a substrate for methylenetetrahydrofolate reductase (MTHFR), but also for thymidylate synthase (TS) and methylenetetrahydrofolate dehydrogenase (MTHFD) enzymes responsible for biosynthesis of thymidine monophosphate (dTMP) and purines, respectively.

 

catalyzes the conversion of SAH, in a reversible reaction, to homocysteine and adenosine. Since the equilibrium constant of SAH hydrolase favors SAH synthesis, the hydrolysis of SAH greatly depends on the efficient removal of homocysteine (Fig. 1).

Homocysteine can be removed via remethylation to methionine in a folate/$B_{12}$-dependent methionine synthase (methylfolate-homocysteine methyltransferase) reaction and/or a betaine-homocysteine methyltransferase reaction. Betaine-homocysteine methyltransferase uses betaine, a metabolite of choline, as the methyl donor for homocysteine methylation. A third route of homocysteine removal is the irreversible transsulfuration pathway, which involves two pyridoxal phosphate-dependent enzymes cystathionine β-synthase and cystathionine γ-lyase. Consequently, continuous homocysteine recycling or removal is necessary for the methionine cycle, choline synthesis, and folate diversion for nucleotide synthesis. Therefore, homocysteine is a key branching point metabolite point for methionine, choline, folate, and vitamin $B_{12}$ and $B_6$ metabolic pathways [30]. Exogenous dietary deficiency in any of these nutrients results in profound dysregulation and imbalance within these several interdependent metabolic processes. Additionally, these metabolic pathways are tightly connected with folate-dependent DNA synthesis, because 5,10-methylenetetrahydrofolate is not only a substrate for methylenetetrahydrofolate reductase, but also for thymidylate synthase and methylenetetrahydrofolate dehydrogenase (Fig. 1), key enzymes that are required for biosynthesis of thymidine monophosphate and purines for DNA synthesis, respectively [31, 32].

The administration of the methyl-deficient diets is generally associated with decreased levels of SAM, an increased content of SAH, and decreased SAM/SAH ratios in the livers of male rats and mice [33–35]. Importantly, these changes occur rapidly and persist in preneoplastic and neoplastic liver tissues. Similar changes have been found with adequate levels of methyl donors during chemically induced liver carcinogenesis [36–39]. The aberrant SAM content may contribute to liver carcinogenesis through multiple mechanisms, including altered cellular methylation reactions, induction of oxidative stress, dysregulated cell proliferation and apoptosis, and altered DNA repair.

## 3 Altered lipid metabolism

In addition to rapid SAM depletion, the initial changes in the livers of rats or mice fed methyl-deficient diets include a rapid – within days of administering the diets – increase in the accumulation of intrahepatic triglycerides [19, 40, 41]. This accumulation has been attributed to increased long-chain fatty acid uptake, increased de novo triglyceride synthesis, compromised de novo lipogenesis, and altered fatty acid β-oxidation [19, 40, 42]. Also, a lack of choline impairs biosynthesis of phosphatidylcholine, which affects lipid export because phosphatidylcholine is a major constituent of very low-density lipoprotein that transports fat from liver to peripheral tissues [40]. Additionally, choline deficiency causes a marked accumulation of 1,2-diradylglycerol [43], a key intermediate in the triglyceride and phosphatidylcholine biosynthesis. 1,2-Diradylglycerol is also an important lipid second messenger involved in proliferative signaling via activation of protein kinase C [44]. Feeding rat a choline-deficient diet causes a rapid and stable increase in hepatic 1,2-diradylglycerol concentration accompanied by a sustained up-regulation of protein kinase C [16, 44, 45]. It is widely believed that these alterations may be major procarcinogenic events induced by choline deficiency.

Feeding methyl-deficient diets results in a significant alteration in expression of numerous genes involved in lipid metabolism [19, 40], including the gene encoding stearoyl-coenzyme A desaturase-1 (*Scd1*). This is one of the most significantly down-regulated genes in the livers of animals exposed to methyl deficiency [40, 46, 47]. Scd1 is a key regulator of intracellular fatty acid composition. This enzyme converts saturated fatty acids into monounsaturated fatty acids, such as palmitoleic and oleic acids, key substrates for the formation of triglycerides, phospholipids, and cholesterol [47, 48]. Therefore, marked down-regulation of the *Scd1* gene may enhance accumulation of saturated free fatty acids and lead to the induction of oxidative stress and lipid peroxidation in the liver.

## 4 Induction of oxidative stress

Dietary methyl deficiency causes the induction of ''oxidative stress'' in the livers of exposed rats and mice. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are necessary for numerous cellular physiologic functions of hepatocytes, including cell signaling, oxidative respiration, microsomal defense, and apoptosis [49]. However, when generation of ROS and/or RNS exceeds the capacity of the cellular antioxidant defense system to control their production oxidative stress arises [50]. Methyl deficiency promotes oxidative stress by several possible mechanisms, including alterations in mitochondrial metabolism, increased production of oxygen/nitrogen free radicals, increased lipid peroxidation, reduction of intracellular and mitochondrial levels of reduced glutathione, depletion of the antioxidant defense system, and altered DNA repair [19, 51–57]. In liver, an excess of ROS and RNS generation causes, directly or indirectly, chemical modifications of DNA, proteins, and lipids. For instance, ROS induce either direct damage to DNA and proteins or indirect damage through alterations in lipid metabolism (e.g. induction of lipid peroxidation), in which metabolites are also damaging agents. These ROS- and RNS-induced chemical modifications of DNA, proteins, and lipids result in their functional inactivation causing

 

damage to all of the cells present in the liver, including hepatocytes, stellate cells, Kupffer cells, and endothelial cells, leading ultimately to the malignant transformation [58].

## 5 DNA damage

One of the main consequences of oxidative stress is an induction of DNA damage that is evidenced by sequential accumulation of 8-oxodeoxyguanosine in DNA and single-strand DNA breaks [59, 60] and the up-regulation of genes involved in the base excision DNA repair pathway [19, 60]. The results of numerous studies have established that feeding methyl-deficient diets results in sequential progressive accumulation of 8-oxodeoxyguanosine in DNA [59] and increased expression of base excision repair genes, including *Ogg1*, *Parp*, *Ape*, and *Pol*β [19, 60]. The over-expression of these genes is considered a sensitive in vivo biomarker of persistent oxidative damage to DNA [61].

Oxidative stress has long been known to contribute to carcinogenesis, mainly via the induction of oxidative damage to DNA, which is widely believed to play a critical role in the initiation of carcinogenesis [62]. However, despite the fact that direct damage to DNA is one of the key events, it is highly unlikely that it is sufficient for cancer development [63]. For example, elevated levels of oxidative DNA lesions are not always associated with increased cancer incidence.

In addition to oxidative DNA lesions, DNA isolated from animals fed methyl-deficient diets, especially those that contain low levels of folic acid or vitamin $B_{12}$, is characterized by a greater level of uracil misincorporation [64, 65]. Folic acid plays a fundamental role in the synthesis of purine ring and de novo thymidylate biosynthesis (Fig. 1). Thymidylate synthase converts deoxyuridine monophosphate to thymidine monophosphate by using 5,10-methylenetetrehydrofolate as a methyl donor. Low dietary folate leads to a decrease in intracellular level of tetrahydrofolates, particularly 5,10-methylenetetrehydrofolate, and, subsequently, to an imbalance in nucleotide precursors for DNA synthesis [66, 67]. The results of numerous comprehensive studies have established that feeding a folate-methyl-deficient diet is associated with the accumulation of deoxyuridine monophosphate and misincorporation of uracil into DNA in place of thymine [65–67] leading consequently to genomic instability, especially under defects in folate metabolism [68].

## 6 Epigenetic alterations

The most widely investigated hypothesis for the carcinogenic properties of methyl-deficient diets is that dietary methyl insufficiency results in variety of cancer-related epigenetic abnormalities.

### 6.1 DNA methylation changes

One of the earliest epigenetic changes during hepatocarcinogenesis induced by diets lacking methyl donors is a sustained demethylation of genomic DNA [13, 15–18] that occurs almost exclusively in the livers of methyl-deficient animals [69]. The results of numerous studies have suggested that this demethylation event is mainly associated with decreased levels of SAM, an increased SAH content, and a decreased SAM/SAH ratio [13], and altered expression/activity of DNA methyltransferases [70]. However, the results of our studies, contrary to previous reports, demonstrated that in addition to the inadequate levels of methyl donors, compromised integrity of DNA induced by methyl-deficient diets is a significant contributor to DNA hypomethylation [60]. This DNA hypomethylation may be attributed to the induction of DNA lesions, specifically 8-oxodeoxyguanosine, uracil, and, especially, 5-hydroxymethylcytosine [17, 71–73]. The presence of these lesions in DNA severely compromises the ability of DNA methyltransferases to methylate the target cytosine and leads to demethylation of DNA [71]. In addition, activation of DNA repair pathway promotes active demethylation of DNA [74].

DNA hypomethylation represents one of the two major DNA methylation states and refers to a relative situation in which there is a decrease from the ''normal'' methylation level. The methylation landscape of mammalian genomes consists of short ($<4$ kb) unmethylated CpG-island-containing domains embedded in a matrix of long stably methylated domains, which are composed predominantly of interspersed and tandem repetitive sequences and exons distal to first exons [75, 76]. A decrease in DNA methylation largely affects only these specific areas of the genome. Hypomethylation of DNA was the first epigenetic abnormality identified in cancer more than a quarter century ago (reviewed in [77]), and it continues to be a central feature and one of the most common molecular alterations in all major human cancers, including liver cancer [78–80]. However, a decrease in DNA methylation, by itself, may not be sufficient to explain its role in tumorigenesis, because loss of DNA methylation could simply be a secondary consequence of malignant cell transformation reflecting the undifferentiated state of tumors. To provide evidence that DNA hypomethylation has a significant role in cancer development, it is necessary to demonstrate that: (i) the loss of DNA methylation occurs at a considerable frequency at early stages of carcinogenesis; (ii) changes occurring at preneoplastic stages are also present during later stages of cancer development; (iii) additional changes in methylation are acquired during tumor progression; and (iv) a mechanistic link exists between hypomethylation of DNA and cancer development.

Numerous studies using methyl-deficient models of hepatocarcinogenesis have demonstrated: (i) the frequent loss of genome-wide DNA methylation in the livers of rats

 

and mice fed methyl-deficient diets occurs rapidly and is associated with the development of preneoplastic foci [41, 69, 81]; (ii) a greater degree of DNA hypomethylation in liver tumors compared with preneoplastic tissues [82, 83]; and (iii) cumulative methylation changes during cancer progression [84]. Together, these studies provide convincing evidence that loss of DNA methylation during hepatocarcinogenesis is not a secondary event.

The mechanistic link between DNA demethylation and cancer development is directly related to alterations in the methylation landscape of mammalian genomes. As mentioned above, loss of DNA methylation largely affects areas of the genome composed of repetitive sequences and exons other than first exons. There are three well-established consequences associated with demethylation of DNA at these regions that may contribute to tumorigenesis. First, demethylation of repetitive sequences located at centromeric, pericentromeric, and subtelomeric chromosomal regions may cause the induction of chromosomal abnormalities. For example, the recent findings have demonstrated that DNA hypomethylation at the centromeric regions causes permissive transcriptional activity at the centromere [85] and the subsequent accumulation of small minor satellite transcripts that impair centromeric architecture and function. Similarly, hypomethylation of the subtelomeric regions is associated with enhanced transcription of the telomeric region [86]. Second, hypomethylation of retroviral mobile repetitive elements causes their activation and transposition [87], which may lead to genomic instability. Third, in addition to loss of repetitive element DNA methylation, methyl-deficient diets may promote liver carcinogenesis by causing demethylation-induced activation of critical proto-oncogenes, including *c-myc*, *c-fos*, and *c-H-ras* [13, 14]. The causal role of these alterations in carcinogenesis is now commonly accepted.

DNA hypermethylation is the alternative state of DNA methylation referring to the relative increase of methylation at normally unmethylated genomic regions, especially at CpG islands. Promoters and first exons of the majority of genes (>60%) in the genome are enriched in unmethylated domains and depleted in methylated domains [75]. In normal cells, CpG sites located in CpG islands are undermethylated, while most of the remaining CpG sites are methylated. Previous studies have demonstrated that feeding rats methyl-deficient diets causes hypermethylation of tumor-suppressor genes, including *p53*, $p16^{INK4a}$, *PtprO*, *Cdh1*, and *Cx26* [88–91]; however, it is unclear whether these hypermethylation events are caused by methyl deficiency or are a consequence of the carcinogenic process. Recently, by using gene expression and CpG island microarrays, we demonstrated that feeding a methyl-deficient diet results in early hypermethylation and transcriptional silencing of a number of genes prior to formation of putative preneoplastic lesions in the livers of mice [92].

### 6.2 Histone modification changes

DNA methylation changes induced by methyl deficiency are not isolated events. They occur in the environment of large-scale disruption of the cellular epigenome and are associated primarily with alteration of histone modifications, especially acetylation, methylation, phosphorylation, biotinylation, and ubiquitylation. These histone marks are essential for organizing higher order chromatin structure, maintaining genome stability, silencing repetitive DNA elements, regulating cell-cycle progression, recognizing DNA damage sites and initiating/allowing DNA repair, and proper expression of genetic information [93, 94].

Similar to alterations in DNA methylation, histone modifications induced by methyl-deficient diets occur on both genome-wide and gene-specific scales. Feeding a methyl-deficient diet results in a rapid and progressive decrease in hepatic histone H3 lysine 9 trimethylation (H3K9me3), histone H3 lysine 9 acetylation (H3K9ac), and histone H4 lysine 20 trimethylation (H4K20me3) and an increase in histone H3 serine10 phosphorylation (H3S10ph) [35, 95, 96]. Among these changes, a decrease in the levels of H4K20me3 is the most pronounced and consistent. Several possible explanations exist for the mechanism of loss trimethylation of H3K9 and H4K20. First, it may be attributed to reduced histone methyltransferase activity as a result of a decreased SAM levels, an increased SAH content, and a decreased SAM/SAH ratio in the livers of rats and mice fed methyl-deficient diets. Second, the loss of histone trimethylation may be related to altered expression of histone methyltransferases or to active demethylation by histone demethylases. Feeding a methyl-deficient diet results in decrease in H3K9 and H4K20 trimethylation that is accompanied by down-regulation of Suv39h1, Prdm2/Riz1, and Suv420h2 histone methyltransferases [96, 97]. Importantly, these changes occur prior to formation of preneoplastic lesions and gradually progress in parallel with the formation of liver tumors. Third, since the trimethylation status of histone H3K9 and histone H4K20 is linked intimately to the degree of DNA methylation, the loss of H3K9me3 and H4K20me3 in the livers may be affected by the loss of DNA methylation induced by methyl deficiency.

Collectively, H4K20me3 and H3K9me3, along with H3K27me3, are among the most prominent marks of constitutive heterochromatin that affect higher order chromatin structure [98–101]. Additionally, histone H4K20me3 is located predominantly in centromeric, pericentromeric, and telomeric regions and plays fundamental role in genomic stability by silencing repetitive elements [99, 102, 103]. Therefore, the loss of H3K9me3 and H4K20me3 markedly compromises genome stability, diminishes the ability of cells to maintain cell-cycle arrest, and severely impairs the viability of cells. Any or all these changes may contribute to initiation and progression of carcinogenic process.

 

### 6.3 MicroRNA alterations

In addition to the importance of gene-specific DNA methylation and histone modification changes in the regulation of gene expression, accumulated findings during the recent years have established a critical role of microRNAs (miRNAs) as negative regulators of gene expression. Currently, there are more than 1200 mammalian miRNAs that can potentially target up to one-third of the protein-coding genes involved in diverse physiological and pathological processes. The first evidence indicating that miRNAs may play an important role in rat hepatocarcinogenesis induced by a methyl-deficient diet was reported by Kutay et al. [104], who showed altered expression of miRNAs, especially decreased expression of microRNA miR-122 in preneoplastic and malignant liver tissues. Since then, a large amount of data have documented a substantial microRNAone deregulation not only in liver tumors induced by methyl deficiency, but also in preneoplastic pathological states linked to hepatocarcinogenic process [105, 106]. The changes in miRNA expression, especially down-regulation of miR-122 and miR-29, and up-regulation of miR-34a, miR-155, and miR-221 are associated with key pathophysiological and pathomorphological features of methyl-deficient liver carcinogenesis, including altered lipid metabolism, induction of oxidative stress, dysregulated cell proliferation and apoptosis, and altered DNA methylation reactions. Interestingly, similar pattern of miRNA expression is specific to preneoplastic liver pathological states (e.g. non-alcoholic steatohepatitis) and full-fledged hepatocellular carcinoma in humans [107, 108].

## 7 Concluding remarks

A large body of evidence indicates that any of the above-mentioned molecular aberrations may be significant in hepatocarcinogenesis induced by methyl deficiency; however, not all aberrations are equally important for the tumorigenic process. Specifically, it is highly unlikely that all of the described changes play a causative role in tumorigenesis. For example, some changes may drive other events that contribute to the formation of a transformed phenotype, while others may be passenger events that accompany the transformation process. In this respect, the identification of alterations that drive and promote cell transformation is crucially important for understanding mechanisms of cancer progression and prevention.

It is well established that for any carcinogen-induced preneoplastic alteration to be considered as neoplastic, it needs to be stably maintained after the initiating factor has been removed. Previously, we and other investigators evaluated the evolution of hepatic molecular alterations induced by methyl deficiency after re-feeding a diet with an adequate level of methyl donors [34, 45, 60]. Only this approach allows discrimination between carcinogenic and non-carcinogenic changes, because only changes that persist after removal of carcinogen are regarded as neoplastic. The results of those studies have demonstrated that only epigenetic alterations, but not metabolic, DNA damage, or other molecular abnormalities persist after removal of the methyl-deficient diet [34, 60]. While these results do not diminish the importance of other changes during carcinogenesis, they signify the important role of epigenetic changes as a driving force in progression of hepatocarcinogenesis.

The unique feature of epigenetic aberrations is their reversibility. This is the foundation for a novel therapeutic approach in cancer treatment termed ''epigenetic therapy'' [109, 110]. However, the timely correction of epigenetic alterations may be even more important for the prevention of cancer development [111]. Accumulating evidence suggests that dietary components may be significant regulators of the cellular epigenome by providing and maintaining the adequate levels of metabolites, such as *S*-adenosylmethionine, flavin adenine dinucleotide, nicotine adenine dinucleotide, acetyl coenzyme A, and α-ketoglutarate, which are essential for normal DNA methylation and various histone modification reactions [112]. Additionally, epigenetic processes may be optimized by efficient functioning of many intracellular metabolic pathways, including transmethylation and transsulfuration pathways. In addition, the accurate maintenance of the cellular redox balance by dietary antioxidants may also assure the normal status of epigenomic homeostasis. This indicates clearly that early identification and timely correction of epigenetic alterations by dietary interventions is a promising avenue for cancer prevention.

*The views expressed in this paper do not necessarily represent those of the U.S. Food and Drug Administration.*

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## 8 References

[1] Michels, K. B., The role of nutrition in cancer development and prevention. *Int. J. Cancer* 2005, *114*, 163–165.

[2] Willett, W., Nutrition and cancer: The search continues. *Nutr. Cancer* 2008, *60*, 557–559.

[3] Milner, J. A., Diet and cancer: facts and controversies. *Nutr. Cancer* 2006, *56*, 216–224.

[4] World Cancer Research Fund/American Institute for Cancer Research, *Food, nutrition, physical activity, and the prevention of cancer: a global perspective*, AICR, Washington, DC, USA 2007.

[5] Ross, S. A., in: Milner, J. A., Romagnolo, D. F. (Eds.), *Bioactive Compounds and Cancer*, Humana Press, Totowa, NJ, USA 2010, pp.101–123.

 

[6] Kaaks, R., Riboli, E., Epidemiologic studies of nutrition and cancer: let us not throw out the baby with the bath water. *Int. J. Cancer* 2005, *116*, 662–664.

[7] Key, T. J., Fruit and vegetables and cancer risk. *Br. J. Cancer* 2001, *104*, 6–11.

[8] McCullough, M. L., Patel, A. V., Kushi, L. H., Patel, R. et al., Following cancer prevention guidelines reduces risk of cancer, cardiovascular disease, and all-cause mortality. *Cancer Epidemiol. Biomarkers Prev.* 2011, *20*, 1089–1097.

[9] Copeland, D. H., Salmon, W. D., The occurrence of neoplasms in the liver, lungs, and other tissues of rats as a result of prolonged choline deficiency. *Am. J. Pathol.* 1946, *22*, 1059–1079.

[10] Lombardi, B., Shinozuka, H., Enhancement of 2-acetylaminofluorene liver carcinogenesis in rats fed a choline-devoid diet. *Int. J. Cancer* 1979, *23*, 565–570.

[11] Mikol, Y. B., Hoover, K. L., Creasia, D., Poirier, L. A., Hepatocarcinogenesis in rats fed methyl-deficient, amino acid-defined diets. *Carcinogenesis* 1983, *4*, 1619–1629.

[12] Newberne, P. M., Rogers, A. E., Labile methyl groups and the promotion of cancer. *Annu. Rev. Nutr.* 1986, *6*, 407–432.

[13] Wainfan, E., Poirier, L. A., Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. *Cancer Res.* 1992, *52*, 2071s–2077s.

[14] Ghoshal, A. K., New insight into the biochemical pathology of liver in choline deficiency. *Crit. Rev. Biochem. Mol. Biol.* 1995, *30*, 263–273.

[15] Christman, J. K., Dietary effects on DNA methylation: do they account for hepatocarcinogenic properties of lipotrope deficient diets? *Adv. Exp. Med. Biol.* 1995, *369*, 141–154.

[16] Zeisel, S. H., da Costa, K. A., Albright, C. D., Shin, O. H., Choline and hepatocarcinogenesis in the rat. *Adv. Exp. Biol. Med.* 1995, *375*, 65–74.

[17] James, S. J., Pogribny, I. P., Pogribna, M., Miller, B. J. et al., Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. *J. Nutr.* 2003, *133*, 3740S–3747S.

[18] Brunaud, L., Alberto, J. M., Ayav, A., Gérard, P. et al., Effects of vitamin $B_{12}$ and folate deficiencies on DNA methylation and carcinogenesis in rat liver. *Clin. Chem. Lab. Med.* 2003, *41*, 1012–1019.

[19] Powell, C. L., Kosyk, O., Bradford, B. U., Parker, J. S. et al., Temporal correlation of pathology and DNA damage with gene expression in a choline-deficient model of rat liver injury. *Hepatology* 2005, *42*, 1137–1147.

[20] Larter, C. Z., Yeh, M. M., Animal models of NASH: Getting both pathology and metabolic context right. *J. Gastroenterol. Hepatol.* 2008, *23*, 1635–1648.

[21] Hebbard, L., George, J., Animal models of nonalcoholic fatty liver disease. *J. Nat. Rev. Gastroenterol. Hepatol.* 2011, *8*, 34–44.

[22] Siegel, A. B., Zhu, A. X., Metabolic syndrome and hepatocellular carcinoma. *Cancer* 2009, *115*, 5651–5661.

[23] Sanyal, A., Poklepovic, A., Moyneur, E., Barghout, V., Population-based risk factors and resource utilization for HCC: UA perspective. *Curr. Med. Res. Opin.* 2010, *26*, 2183–2191.

[24] Cantoni, G. L., Biological methylation: Selected aspects. *Annu. Rev. Biochem.* 1975, *44*, 435–451.

[25] Mato, J. M., Lu, S. C., Role of *S*-adenosyl-L-methionine in liver health and injury. *Hepatology* 2007, *45*, 1306–1312.

[26] Mato, J. M., Alvarez, L., Ortiz, P., Pajares, M. A., *S*-Adenosylmethionine synthesis: molecular mechanisms and clinical implications. *Pharmacol. Ther.* 1997, *73*, 265–280.

[27] Lu, S. C., *S*-Adenosylmethionine. *Int. J. Biochem. Cell. Biol.* 2000, *32*, 391–395.

[28] Yi, P., Melnyk, S., Pogribna, M., Pogribny, I. P. et al., Increase in plasma homocysteine associated with parallel increases in plasma *S*-adenosylhomocysteine and lymphocyte DNA hypomethylation. *J. Biol. Chem.* 2000, *275*, 29318–29323.

[29] Finkelstein, J. D., Metabolic regulatory properties of *S*-adenosylmethionine and *S*-adenosylhomocysteine. *Clin. Chem. Lab. Med.* 2007, *45*, 1694–1699.

[30] Niculescu, M. D., Zeisel, S. H., Diet, methyl donors and DNA methylation: interactions between dietary folate, methionine and choline. *J. Nutr.* 2002, *132*, 2333S–2335S.

[31] Duthie, S. J., Folic acid deficiency and cancer: mechanisms of DNA instability. *Br. Med. Bull.* 1999, *55*, 578–599.

[32] Stover, P. J., One-carbon metabolism-genome interactions in folate-associated pathologies. *J. Nutr.* 2009, *139*, 2402–2405.

[33] Shivapurkar, N., Poirier, L. A., Tissue levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine in rats fed methyl-deficient, amino-defined diets for one to five weeks. *Carcinogenesis* 1983, *4*, 1051–1057.

[34] Pogribny, I. P., Ross, S. A., Wise, C., Pogribna, M. et al., Irreversible global DNA hypomethylation as a key step in hepatocarcinogenesis induced by dietary methyl deficiency. *Mutat. Res.* 2006, *593*, 80–87.

[35] Pogribny, I. P., Tryndyak, V. P., Bagnyukova, T. V., Melnyk, S. et al., Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. *J. Hepatol.* 2009, *51*, 176–186.

[36] Simile, M. M., Pascale, R., De Miglio, M. R., Nufris, A. et al., Correlation between *S*-adenosyl-L-methionine content and production of c-myc, c-Ha-ras, and c-Ki-ras mRNA transcripts in the early stages of rat liver carcinogenesis. *Cancer Lett.* 1994, *79*, 9–16.

[37] Huang, Z. Z., Mato, J. M., Kanel, G., Lu, S. C., Differential effect of thioacetamide on hepatic methionine adenosyltransferase expression in the rat. *Hepatology* 1999, *29*, 1471–1478.

[38] Pascale, R. M., Simile, M. M., De Miglio, M. R., Feo, F., Chemoprevention of hepatocarcinogenesis: *S*-adenosyl-L-methionine. *Alcohol* 2002, *27*, 193–198.

 

[39] Calvisi, D. F., Simile, M. M., Ladu, S., Pellegrino, R. et al., Altered methionine metabolism and global DNA methylation in liver cancer: relationship with genomic instability and prognosis. *Int. J. Cancer* 2007, *121*, 2410–2420.

[40] Rinella, M. E., Elias, M. S., Smolak, R. R., Fu, T. et al., Mechanisms of hepatic steatosis in mice fed a lipogenic methionine choline-deficient diet. *J. Lipid. Res.* 2008, *49*, 1068–1076.

[41] Pogribny, I. P., Tryndyak, V. P., Bagnyukova, T. V., Melnyk, S. et al., Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. *J. Hepatol.* 2009, *51*, 176–186.

[42] Larter, C. Z., Yeh, M. M., Haigh, W. G., Williams, J. et al., Hepatic free fatty acids accumulate in experimental steatohepatitis: role of adaptive pathways. *J. Hepatol.* 2008, *48*, 638–647.

[43] Blusztajn, J. K., Zeisel, S. H., 1,2-*sn*-Diacylglycerol accumulates in choline-deficient liver. A possible mechanism of hepatic carcinogenesis via alteration in protein kinase C activity? *FEBS Lett.* 1989, *243*, 267–270.

[44] da Costa, K. A., Cochary, E. F., Blusztajn, J. K., Garner, S. C. et al., Accumulation of 1,2-*sn*-diradylglycerol with increased membrane-associated protein kinase C may be the mechanism for spontaneous hepatocarcinogenesis in choline-deficient rats. *J. Biol. Chem.* 1993, *268*, 2100–2105.

[45] da Costa, K. A., Garner, S. C., Chang, J., Zeisel, S. H., Effects of prolonged (1 year) choline deficiency and subsequent re-feeding of choline on 1,2-*sn*-diradylglycerol, fatty acids and protein kinase C in rat liver. *Carcinogenesis* 1995, *16*, 327–334.

[46] Rizki, G., Arnaboldi, L., Gabrielli, B., Yan, J. et al., Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. *J. Lipid Res.* 2006, *47*, 2280–2290.

[47] Gornicka, A., Morris-Stiff, G., Thapaliya, S., Papouchado, B. G. et al., Transcriptional profile of genes involved in oxidative stress and antioxidant defense in a dietary murine model of steatohepatitis. *Antioxid. Redox Signal.* 2011, *15*, 437–445.

[48] Flowers, M. T., Ntambi, J. M., Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. *Curr. Opin. Lipidol.* 2008, *19*, 248–256.

[49] Diesen, D. L., Kuo, P. C., Nitric oxide and redox regulation in the liver: part I. General considerations and redox biology in hepatitis. *J. Surg. Res.* 2010, *162*, 95–109.

[50] Winyard, P. G., Moody, C. J., Jacob, C., Oxidative activation of antioxidant defence. *Trends Biochem. Sci.* 2005, *30*, 453–461.

[51] Hensley, K., Kotake, Y., Sang, H., Pye, Q. N. et al., Dietary choline restriction causes complex I dysfunction and increased $H_2O_2$ generation in liver mitochondria. *Carcinogenesis* 2000, *21*, 983–989.

[52] Romestaing, C., Piquet, M. A., Letexier, D., Rey, B. et al., Mitochondrial adaptations to steatohepatitis by a methionine- and choline-deficient diet. *Am. J. Physiol. Endocrinol. Metab.* 2008, *294*, E110–E119.

[53] Leclercq, I. A., Farrell, G. C., Field, J., Bell, D. R. et al., CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J. Clin. Invest.* 2000, *105*, 1067–1075.

[54] Yoshida, Y., Itoh, N., Hayakawa, M., Habuchi, Y. at el., Lipid peroxidation in mice fed a choline-deficient diet as evaluated by total hydroxyoctadecadienoic acid. *Nutrition* 2006, *22*, 303–311.

[55] Henning, S. M., Swenseid, M. E., Ivandic, B. T., Liao, F., Vitamins C, E and A and heme oxygenase in rats fed methyl/folate-deficient diets. *Free Radic. Biol. Med.* 1997, *23*, 936–942.

[56] Caballero, F., Fernández, A., Matias, N., Martinez, L. et al., Specific contribution of methionine and choline in nutritional nonalcoholic steatohepatitis: impact on mitochondrial *S*-adenosyl-L-methionine and glutathione. *J. Biol. Chem.* 2010, *285*, 18528–18536.

[57] Fujita, K., Nozaki, Y., Yoneda, M., Wada, K. et al., Nitric oxide plays a crucial role in the development/progression of nonalcoholic steatohepatitis in the choline-deficient, L-amino acid-defined diet-fed rat model. *Alcohol Clin. Exp. Res.* 2010, *34*, S18–S24.

[58] Diesen, D. L., Kuo, P. C., Nitric oxide and redox regulation in the liver: part II. Redox biology in pathologic hepatocytes and implications for intervention. *J. Surg. Res.* 2011, *167*, 96–112.

[59] Nakae, D., Endogenous liver carcinogenesis in the rat. *Pathol. Int.* 1999, *49*, 1028–1042.

[60] Pogribny, I. P., Shpyleva, S. I., Muskhelishvili, L., Bagnyukova, T. V. et al., Role of DNA damage and alterations in cytosine DNA methylation in rat liver carcinogenesis induced by a methyl-deficient diet. *Mutat. Res.* 2009, *669*, 56–62.

[61] Powell, C. L., Swenberg, J. A., Rusyn, I., Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. *Cancer Lett.* 2005, *229*, 1–11.

[62] Ziech, D., Franco, R., Pappa, A., Panayiotidis, M. I., Reactive oxygen species (ROS)-induced genetic and epigenetic alterations in human carcinogenesis. *Mutat. Res.* 2011, *711*, 167–173.

[63] Halliwell, B., Oxidative stress and cancer: Have we moved forward? *Biochem. J.* 2007, *401*, 1–11.

[64] Pogribny, I. P., Muskhelishvili, L., Miller, B. J., James, S. J., Presence and consequence of uracil in preneoplastic DNA from folate/methyl-deficient rats. *Carcinogenesis* 1997, *18*, 2071–2076.

[65] Duthie, S. J., Narayanan, S., Brand, G. M., Pirie, L. et al., Impact of folate deficiency on DNA stability. *J. Nutr.* 2002, *132*, 2444S–2449S.

[66] James, S. J., Cross, D. R., Miller, B. J., Alterations in nucleotide pools in rats fed diets deficient in choline, methionine, and/or folic acid. *Carcinogenesis* 1992, *13*, 2471–2474.

[67] James, S. J., Miller, B. J., Basnakian, A. G., Pogribny, I. P. et al., Apoptosis and proliferation under conditions of deoxynucleotide pool imbalance in liver of folate/methyl deficient rats. *Carcinogenesis* 1997, *18*, 287–293.

 

[68] Berger, S. H., Pittman, D. L., Wyatt, M. D., Uracil in DNA: Consequences for carcinogenesis and chemotherapy. *Biochem. Pharmacol.* 2008, *76*, 697–706.

[69] Pogribny, I. P., James, S. J., Jernigan, S., Pogribna, M., Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues. *Mutat. Res.* 2004, *548*, 53–59.

[70] Ghoshal, K., Li, X., Datta, J., Bai, S. et al., A folate-and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. *J. Nutr.* 2006, *136*, 1522–1527.

[71] Valinluck, V., Sowers, L. C., Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. *Cancer Res.* 2007, *67*, 946–950.

[72] Dahl, C., Grønbæk, K., Guldberg, P., Advances in DNA methylation: 5-hydroxymethylcytosine revisited. *Clin. Chim. Acta* 2010, *412*, 831–836.

[73] Chia, N., Wang, L., Lu, X., Senut, M. C. et al., Hypothesis: environmental regulation of 5-hydroxymethylcytosine by oxidative stress. *Epigenetics* 2011, *6*, 853–856.

[74] Ma, D. K., Guo, J. U., Ming, G. L., Song, H., DNA excision repair proteins and Gadd45 as molecular players for active DNA demethylation. *Cell Cycle* 2009, *8*, 1526–1531.

[75] Rollins, R. A., Haghighi, F., Edwards, J. R., Das, R. et al., Large-scale structure of genomic methylation patterns. *Genome Res.* 2006, *16*, 157–163.

[76] Suzuki, M. M., Bird, A., DNA methylation landscapes: provocative insights from epigenomics. *Nat. Rev. Genet.* 2008, *9*, 465–476.

[77] Feinberg, A. P., Tycko, B., The history of cancer epigenetics. *Nat. Rev. Cancer* 2004, *4*, 143–153.

[78] Guerrero-Preston, R., Santella, R. M., Blanco, A., Desai, M. et al., Global DNA hypomethylation in liver cancer cases and controls: a phase I preclinical biomarker development study. *Epigenetics* 2007, *2*, 223–226.

[79] Kanai, Y., Genome-wide DNA methylation profiles in precancerous conditions and cancers. *Cancer Sci.* 2010, *101*, 36–45.

[80] Stefanska, B., Huang, J., Bhattacharyya, B., Suderman, M. et al., Definition of the landscape of promoter DNA hypomethylation in liver cancer. *Cancer Res.* 2011, *71*, 5891–5903.

[81] Steinmetz, K. L., Pogribny, I. P., James, S. J., Pitot, H. C., Hypomethylation of the rat glutathione-*S*-transferase pi (GSTP) promoter region isolated from methyl-deficient livers and GSTP-positive liver neoplasms. *Carcinogenesis* 1998, *19*, 1487–1494.

[82] Asada, K., Kotake, Y., Asada, R., Saunders, D. et al., LINE-1 hypomethylation in a choline-deficiency-induced liver cancer in rats: dependence on feeding period. *J. Biomed. Biotechnol.* 2006, *2006*, 17142.

[83] Pogribny, I. P., Miller, B. J., James, S. J., Alterations in hepatic P53 gene methylation patterns during tumor progression with folate/methyl deficiency in the rat. *Cancer Lett.* 1997, *115*, 31–38.

[84] Tryndyak, V., Kovalchuk, O., Pogribny, I. P., Identification of differentially methylated sites within unmethylated DNA domains in normal and cancer cells. *Anal. Biochem.* 2006, *356*, 202–207.

[85] Wong, N. C., Wong, L. H., Quach, J. M., Canham, P. et al., Permissive transcriptional activity at the centromere through pockets of DNA hypomethylation. *PLoS Genet.* 2006, *2*, e17.

[86] Vera, E., Canela, A., Fraga, M. F., Esteller, M. et al., Epigenetic regulation of telomeres in human cancer. *Oncogene* 2008, *27*, 6817–6833.

[87] Howard, G., Eiges, R., Gaudet, F., Jaenisch, R. et al., Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. *Oncogene* 2008, *27*, 404–408.

[88] Pogribny, I. P., Pogribna, M., Christman, J. K., James, S.J., Single-site methylation within the p53 promoter region reduces gene expression in a reporter gene construct: possible in vivo relevance during tumorigenesis. *Cancer Res.* 2000, *60*, 588–594.

[89] Pogribny, I. P., James, S. J., De novo methylation of the p16INK4A gene in early preneoplastic liver and tumors induced by folate/methyl deficiency in rats. *Cancer Lett.* 2002, *187*, 69–75.

[90] Motiwala, T., Ghoshal, K., Das, A., Majumder, S. et al., Suppression of the protein tyrosine phosphatase receptor type O (PTPRO) by methylation in hepatocellular carcinomas. *Oncogene* 2003, *22*, 6319–6331.

[91] Tsujiuchi, T., Shimizu, K., Itsuzaki, Y., Onishi, M. et al., CpG site hypermethylation of *E*-cadherin and Connexin26 genes in hepatocellular carcinomas induced by a choline-deficient L-amino-acid-defined diet in rats. *Mol. Carcinog.* 2007, *46*, 269–274.

[92] Tryndyak, V. P., Han, T., Muskhelishvili, L., Fuscoe, J. C. et al., Coupling global methylation and gene expression profiles reveal key pathophysiological events in liver injury induced by a methyl-deficient diet. *Mol. Nutr. Food. Res.* 2011, *55*, 411–418.

[93] Kouzarides, T., Chromatin modifications and their function. *Cell* 2007, *128*, 693–705.

[94] Bannister, A. J., Kouzarides, T., Regulation of chromatin by histone modifications. *Cell Res.* 2011, *21*, 381–395.

[95] Pogribny, I. P., Ross, S. A., Tryndyak, V. P., Pogribna, M. et al., Histone H3 lysine 9 and H4 lysine 20 trimethylation and the expression of Suv4-20h2 and Suv-39h1 histone methyltransferases in hepatocarcinogenesis induced by methyl deficiency in rats. *Carcinogenesis* 2006, *27*, 1180–1186.

[96] Pogribny, I. P., Tryndyak, V. P., Muskhelishvili, L., Rusyn, I. et al., Methyl deficiency, alterations in global histone modifications, and carcinogenesis. *J. Nutr.* 2007, *137*, 216S–222S.

[97] Zhou, W., Alonso, S., Takai, D., Lu, S. C. et al., Requirement of RIZ1 for cancer prevention by methyl-balanced diet. *PLoS One* 2008, *3*, e3390.

[98] Kourmouli, N., Jeppesen, P., Mahadevhaiah, S., Burgoyne, P. et al., Heterochromatin and tri-methylated lysine 20 of histone H4 in animals. *J. Cell. Sci.* 2004, *117*, 2491–2501.

 

[99] Schotta, G., Lachner, M., Sarma, K., Ebert, A. et al., A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev.* 2004, *18*, 1251–1262.

[100] Jenuwein, T., The epigenetic magic of histone lysine methylation. *FEBS J.* 2006, *273*, 3121–3135.

[101] Black, J. C., Whetstine, J. R., Chromatin landscape: methylation beyond transcription. *Epigenetics* 2011, *6*, 9–15.

[102] Mikkelsen, T. S., Ku, M., Jaffe, D. B., Issac, B. et al., Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* 2007, *448*, 553–560.

[103] Rosenfeld, J. A., Wang, Z., Schones, D. E., Zhao, K. et al., Determination of enriched histone modifications in non-genic portions of the human genome. *BMC Genomics* 2009, *10*, 143.

[104] Kutay, H., Bai, S., Datta, J., Motiwala, T. et al., Down-regulation of miR-122 in the rodent and human hepatocellular carcinomas. *J. Cell. Biochem.* 2006, *99*, 671–678.

[105] Wang, B., Majumder, S., Nuovo, G., Kutay, H. et al., Role of microRNA-155 at early stages of hepatocarcinogenesis induced by choline-deficient and amino acid-defined diet in C57BL6 mice. *Hepatology* 2009, *50*, 1152–1161.

[106] Pogribny, I. P., Starlard-Davenport, A., Tryndyak, V.P., Han, T. et al., Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155m and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab. Invest.* 2010, *90*, 1437–1446.

[107] Cheung, O., Puri, P., Eicken, C., Contos, M. J. et al., Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. *Hepatology* 2008, *48*, 1810–1820.

[108] Gramantieri, L., Fornari, F., Callegari, E., Sabbioni, S. et al., MicroRNA involvement inhepatocellular carcinoma. *J. Cell. Mol. Med.* 2008, *12*, 2189–2204.

[109] Issa, J. P., Kantarjian, H. M., Targeting DNA methylation. *Clin. Cancer Res.* 2009, *15*, 3938–3946.

[110] Yang, X., Lay, F., Han, H., Jones, P. A., Targeting DNA methylation for epigenetic therapy. *Trends Pharmacol. Sci.* 2010, *31*, 536–546.

[111] Huang, Y. W., Kuo, C.T., Stoner, K., Huang, T. H. et al., An overview of epigenetics and chemoprevention. *FEBS Lett.* 2011, *585*, 2129–2136.

[112] Teperino, R., Schoonjans, K., Auwerx, J., Histone methyl transferases and demethylases; can they link metabolism and transcription? *Cell Metab.* 2010, *12*, 321-327.

 