**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 575**

**Columns: TOPIC HIGHLIGHT**

Jung Eun Lee, ScD, *Series editor*

**Current trends in the development and application of molecular technologies for cancer epigenetics**

Jang H *et al*.Application of epigenetic technology in cancer

**Hyeran Jang, Hyunjin Shin**

**Hyeran Jang,** Section of Endocrinology, Boston University School of Medicine, Boston, MA 02118, United States

**Hyunjin Shin,** Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Harvard School of Public Health, Boston, MA 02215, United States

**Author contributions:** Jang H and Shin H contributed equally to this work.

**Correspondence to: Hyunjin Shin, PhD,** Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Harvard School of Public Health, Boston, MA 02215, United States. junglee@sm.ac.kr

**Telephone:** +1-617-5518730 **Fax:** +1-617-5517871

**Received:** September 19, 2012 **Revised:** January 7, 2013

**Accepted:** January 29, 2013

**Published online:**

**Abstract**

Current progress in epigenetic research supports the view that diet and dietary components are important in cancer etiology by enhancing or inhibiting carcinogenesis. Since diet and dietary factors may significantly contribute to the causation and progression of many cancers, it is important to find the molecular mechanisms of action of such dietary factors for cancer prevention and treatment. Recently, the role of epigenetic mechanisms in the cancer development and progression has attracted more attention as additional evidence along with traditional DNA sequence based mechanisms such as mutations and structural re-arrangements. Such an increasing interest in cancer epigenetics has also accelerated the development and application of molecular assays and tools for DNA methylation detection and histone modification enrichment analysis. In this paper, key assays and methods for epigenetic research are reviewed and discussed in terms of their utility and usability. In addition, more advanced methods for genome-wide analysis are introduced as part of upcoming research trends and directions.

© 2013 Baishideng. All rights reserved.

**Key words:** Cancer; Epigenetic; Technology; Histone modifications; Diet; Methyl sources; Butyrate

**Core tip:** Recently, cancer epigenetics attracts scholarly attention due to the growing evidence of its importance in cancer development, progression, and possibly treatment. Also, related molecular technologies are also fast evolving, particularly along with the emergence of next-generation sequencing. We wrote this article in order to help readers grasp the cutting-edge trends in cancer epigenetic research and have a scientific perspective on the possible choices of proper molecular techniques for their own related research.

Jang H, Shin H. Current trends in the development and application of molecular technologies for cancer epigenetics.

**Available from**

**DOI:**

**INTRODUCTION**

Diet and dietary factors play an important role in many biological processes and are also involved in the regulation of pathological progressions including cancers. Several epidemiological and preclinical studies suggested that increased intake of bioactive dietary components may modulate cancer risk. Many studies provide compelling evidence that part of the anti-cancer properties contributed to several bioactive dietary components may relate to modulation of epigenetic process including DNA methylation and histone protein modifications. Here, we provide a brief overview of dietary modulation of DNA methylation and histone modifications and its potential role in cancer prevention. Also, we will discuss several new epigenetic methods to help understand the effect of dietary factors on epigenetic modifications.

**EPIGENETICS AND CANCER**

The growing interest in the role of epigenetics in cancer came from the demonstration that epigenetic modifications are involved in tumor development and progression. Epigenetics can be defined as phenomena that alter the expression of the information in the genome at the transcriptional, translational, or posttranslational level without change in DNA sequence[[1](#_ENREF_1),[2](#_ENREF_2)]. Epigenetic information is maintained to preserve cellular identity in normal cells, while cancer cells are characterized by profound alteration of epigenetic regulation[[3-7](#_ENREF_3)]. The overall disruption of epigenetic phenomena is a common feature of all human tumors and includes alteration of DNA methylation and histone modification patterns[[8](#_ENREF_8)]. DNA methylation patterns of neoplastic cells have been recognized as being substantially altered compared with normal cells[[3](#_ENREF_3),[4](#_ENREF_4)]. Two types of changes in the DNA methylation pattern can occur in cancer: global DNA hypomethylation and hypermethylation of CpG islands which are associated with gene silencing[[3](#_ENREF_3),[4](#_ENREF_4),[7](#_ENREF_7)]. DNA in eukaryotic cells is intimately associated with a family of small, basic histone proteins forming a highly ordered and condensed DNA-protein complex termed chromatin. Because of this chromatin structure, changes in DNA methylation in cancer cells are not isolated events; they occur in the context of more complex epigenetic deregulation[[9](#_ENREF_9)]. Chromatin is the physiological template of the genetic information and is composed of DNA, histones, and other chromosomal proteins. The fundamental repeating unit of chromatin is the nucleosome octamer, which consists of 147 base pairs of DNA wrapped around 2 copies each of histones H2A, H2B, H3, and H4[[10](#_ENREF_10)]. The amino-terminal tails of histones are subject to posttranslational modifications, including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation[[11](#_ENREF_11)], and multiple histone modifications may occur on a given histone tail[[12](#_ENREF_12)]. Histone modifications patterns distinguish the structure of chromatin status, in particular, acetylation of histone H3 and H4 is associated with active gene expression with open chromatin structure. Histone acetylation is regulated by several enzymes such as histone acetyltransferase and histone deacetylases activity. Aberrations in post-translational modifications of histones have been shown to occur in cancer cells. Although alterations in global histone modification patterns in cancer cells have remained unknown, recent studies on global histone modifications at specific amino acids have been suggested as predictive clinical outcomes for various cancers[[13-15](#_ENREF_13)]. Additionally, a number of studies have been focused only on changes of a particular histone modification at individual gene promoters in cancer cells.

**DNA METHYLATION**

***Methyl-deficient diet induced hepatocarcinogenesis***

In last 4 decades, researchers have developed various tools for exploring DNA methylation, and started to apply those new technologies to the field of nutrition science. The methyl-deficient model of endogenous hepatocarcinogenesis is one of which in DNA methylation has been extensively studied. This animal model is unique in that dietary omission rather than chemical carcinogens addition can lead to tumor formation[[16](#_ENREF_16)]. Specifically, deficiency of the major dietary sources of methyl groups – methionine, choline, folic acid and vitamin B12 – leads to the development of liver cancers in rats and certain mouse strains[[17-19](#_ENREF_17)]. From early 1990, these animal models have shown that the methyl-deficiency is associated with several defects, including genome-wide DNA hypomethylation and gene-specific hypermethylation[[20-22](#_ENREF_20)]. Importantly, the aberrant epigenetic alterations imposed by this diet have been hypothesized to be the primary mechanism responsible for malignant transformation of rat liver cells[[15](#_ENREF_15),[20](#_ENREF_20),[22](#_ENREF_22),[23](#_ENREF_23)]. Figure 1 displays a simplified version of biological methylation pathway from one-carbon metabolism, emphasizing that various dietary methyl sources (methionine, choline, various co-enzymatic forms of folate and vitamin B2, B6 and B12) play an important roles in DNA methylation. Methyl source deficiency has marked effects on the flow of one-carbon units through this web of reactions as the effect of methyl source deficiency are highlighted in red.The major effect observed in methyl deficiemcy models is a rapid decrease in hepatic S-adenosylmethionine (SAdoMet) levels and genomic DNA hypomethylation. In other recent studies examining the early stages of hepatocarcinogenesis induced by methyl deficiency in rats, substantial alterations in other aspects of the epigenetic machinery have been observed, including aberrant expression of DNA methyltransferases and methyl-CpG binding proteins[[24](#_ENREF_24)], defects in histone methyltransferase protein expression and histone posttranslational modifications[[15](#_ENREF_15)]. In Table 1, various DNA methylation assays were summarized in methyl-deficient model of hepatocarcinogenesis in rodents.

***Genomic DNA methylation assays***

One of widely used methods for global DNA methylation assay is a radioassay that utilizes the enzyme SssI DNA methyltransferase to catalyze the de novo methylation of the CpG sites with radiolabeled [3H]-SAdoMet, a universal methyl donor *in vitro*[[20](#_ENREF_20), [25-28](#_ENREF_25)]. And another method was developed thanks to the discovery of methylation-sensitive restriction endonucleases. In 1999, Pogribny *et al*[[29](#_ENREF_29)] developed a new method based on methylation-sensitive endonucleases followed by single nucleotide extension with radiolabeled [3H]-dCTP. This cytosine extension assay was used in various studies of methyl-deficient model of hepatocarcinogenesis for genomic DNA methylation[[22](#_ENREF_22),[23](#_ENREF_23),[29](#_ENREF_29),[30](#_ENREF_30)]. These enzyme based methods have wide variations in precision as a result of inconsistency in the activity of methyl-sensitive endonucleases and the instability of methyltransferase activity[[31](#_ENREF_31)]. In 2002, Friso *et al*[[32](#_ENREF_32)] developed a method for quantitative determination of 5-methyl-2’deoxycytidine using liquid chromatography/electrospray ionization mass spectrometry (LC/ESI/MS). This method allows accurate measurement of the absolute amount of 5-methyl-2’deoxycytidine relative to the total amount of cytosine residues, furthermore, it requires relatively lower amount of DNA and has a shorter run time for each sample than other HPLC-based methods[[33-35](#_ENREF_33)]. DNA methylation assay by LC/ESI/MS has been widely used for quantitative DNA methylation in animal studies and population-based studies in the light of its greater reproducibility and precision in large number of samples[[32](#_ENREF_32),[36-40](#_ENREF_36)].

***Gene-specific DNA methylation measurements***

DNA methylation has long been recognized as an important factor on the silencing of genes, therefore it has become important to know the methylation status of individual CpG site. The first generation of DNA methylation detection assay is Southern blot or PCR amplification that follows the enzyme digestion with methylation-sensitive restriction endonucleases[[41-46](#_ENREF_41)]. Currently, the most commonly used methods for gene-specific DNA methylation can be categorized into three major methods.

**Bisulfite DNA sequencing and methylation-specific PCR:** Treatment of DNA with bisulfite converts cytosine residue to uracil, but leaves 5-methylcytosine residue unaffected. Bisulfite sequencing involves chemical conversion of cytosine to uracil, followed by PCR, and DNA sequencing[[47](#_ENREF_47)]. While providing single-base resolution, the high cost and labor-intensive steps limit the use of this method for high-throughput analyses[[48](#_ENREF_48)]. MS-PCR also employs bisulfite conversion, but avoids the need to sequence the area of interest. Instead, methyl-specific and unmethyl-specific primer sets are designed, to distinguish methylated from unmethylated DNA in bisulfate-converted DNA[[49](#_ENREF_49)]. This method is powerful to explore CpG islands with high methylation density, as increased numbers of CpG in the primer increase the specificity of the assay. However, these two methods using bisulfite conversion are not currently suitable for whole-genome analysis on multiple samples but commonly used for data validation from array-based methods.

**Methods that focus specific single-CpG:** These include Combined Bisulfite Restriction Analysis (COBRA)[[50](#_ENREF_50)], MethyLight[[51](#_ENREF_51)], and bisulfite pyrosequencing[[52](#_ENREF_52)]. In COBRA, the combination of bisulfite conversion and PCR amplification is used, therefore it results in sequence conversion (unmethylated cytosine residue to thymidine and methylated cytosine to cytosine) which can lead to new methylation-dependent restriction enzyme sites. The following digestion of the PCR product with at least one CpG site in the recognition sequence only proceeds if the CpG site is protected from bisulfite conversion by methylation. For this reason, the signal ratio of restriction products indicating methylation to undigested PCR product representing unmethylated sequences can be used as a measure for the methylation level of this specific CpG. MethyLight is a bisulfite-dependent, fluorescence-based, quantitative real-time PCR method for DNA methylation. MethyLight relies on methylation-specific priming combined with methylation-specific fluorescent probing. This combination of methylation-specific detection principles results in a highly methylation-specific detection technology, with an accompanying ability to sensitively detect very low frequencies of hypermethylated alleles. Bisulfite pyrosequencing has been used to analyze bisulfite-converted DNA without using methylation-specific PCR. Following PCR amplification of the region of interest, pyrosequencing is used to determine the bisulfite-converted sequence of specific CpG sites in the region. The ratio of cytosine to thymidine at individual sites can be determined quantitatively based on the amount of cytosine and thymidine incorporation during the sequence extension. While the methods mentioned above are sensitive, specific, and relatively inexpensive, none of these methods is suitable for analysis of the whole genome, which includes about 28 million CpGs.

**Microarray-based methods:** These enable to interrogate larger numbers of CpG, there are three major types of microarray-based methylation analysis. Direct hybridization to CpG island arrays is the first high-throughput approach capable of detecting DNA methylaion in genes across several CpG sites. Based on the bisulfite modification of DNA, this method utilizes methylation-specific oligonucleotides arrayed on glass slides for detection of all possible methylation in target DNA[[53](#_ENREF_53)]. Methylated DNA immunoprecipitation (MeDIP) is also a large-scale, genome-wide purification method that is used to enrich for methylated DNA sequence using antibody raised against 5-methylcytosine[[54](#_ENREF_54)]. DNA from MeDIP can be used for either array-based hybridization (MeDIP-chip) or high-throughput sequencing (MeDIP-seq). Although MeDIP helps generate comprehensive DNA methylation profiles, both applications have their typical limitation of array-based technology, restricted resolution. The HELP assay (HpaII tiny fragment enrichment by ligation-mediated PCR) is comparative isoschizomer profiling of DNA methylation[[55](#_ENREF_55)]. DNA is digested by HpaII in parallel with MspI (resistant to DNA methylation), and then the HpaII and MspI products are either amplified by ligation-mediated PCR and hybridized using separate fluorochromes to a customized array, or directly sequenced[[56](#_ENREF_56)]. These high-throughput array-based approaches for DNA methylation are relatively inexpensive tool suitable for genome-wide analysis, therefore, help to target aberrant methylation patterns in various cancer models. Furthermore, methylation profiling achieved from high throughput methods will offer differentially methylated regions to understand the effect of dietary factors on epigenetic modifications in cancer, subsequently, provide insight in prevention strategies to reduce the burden of cancer.

**HISTONE DEACETYLASE (HDAC) INHIBITION BY BUTYRATE**

In addition to the effects on DNA methylation, dietary components can affect posttranslational modifications of histones. The dietary agent best studied in histone modifications is the short chain fatty acid butyrate which is generated in the colon as a result of bacterial fermentation of dietary fiber. Higher intake of dietary fiber is associated with reduced risk of colorectal cancer[[57](#_ENREF_57),[58](#_ENREF_58)]. The molecular mechanisms underlying this anti-cancer effect of dietary fiber are poorly understood, however, the strongest evidence is based on the anti-carcinogenic actions of butyrate. Butyrate can be found at millimolar concentrations in the lumen of the colon[[59](#_ENREF_59)], and has inhibitory effects on type I and II HDAC enzymes. Butyrate-induced alterations in histone marks, especially acetylation at histone H3 and/or H4, have been associated with several processes, including cellular differentiation[[60](#_ENREF_60), [61](#_ENREF_61)], cell cycle arrest[[62-64](#_ENREF_62)], apoptosis[[65-67](#_ENREF_65)], and inhibition of invasion[[68](#_ENREF_68)] in a number of cancer cell studies. Table 2 summarized some of evidence of the effects of butyrate on histone acetylation. Although butyrate has strong marks on histone acetylation, a small fraction of cellular genes is regulated in response to butyrate[[69-71](#_ENREF_69)]. Therefore, it should be noted that site-specific approach by Chromatin Immunoprecipitation (ChIP) based experimental tools will provide a better understanding on the chemopreventive effects of butyrate, showing gene-specific histone acetylation and its associated gene expression.

***Global histone acetylation assays***

The first estimates for the rate of acetylation turnover were measured by pulse, pulse-chase, and steady-state acetylation labeling in hepatoma tissue culture cells in 1975[[72](#_ENREF_72)]. Boffa *et al*[[73](#_ENREF_73)] showed that sodium butyrate suppressed histone deacetylation *in vivo* and *in vitro* by measuring the kinetics of [3H] acetate release from histone proteins. Since specific antibodies to modified histones were developed, Western blot has been used to detect histone modifications. As shown in Table 2, butyrate-induced histone acetylation was confirmed by Western blot in many studies.

***Gene-specific histone acetylation measurements***

**Chromatin Immunoprecipitation:** The antibodies to acetylated histone H3 and H4 have been used for ChIP to determine histone acetylation in specific regions of gene promoter and other regulatory regions. ChIP is a specialized immunoprecipitation used to detect the covalent interaction between the DNA sequence and DNA-binding proteins such as transcription factors or histone proteins. ChIP using histone antibodies is able to determine the specific location in the genome that various histone modifications are associated with, indicating the target of the histone modifiers[[74](#_ENREF_74)]. For example, ChIP experiment unveiled that butyrate induced an increase in histone H3 and H4 acetylation within the CDKN1A promoter, which regulates the p21 protein, in Colo-320 human colon cancer cells[[64](#_ENREF_64)]. Due to its ability to precisely detect the DNA binding of modified histones, transcription factors, and non-histone chromosomal proteins, ChIP has been widely used to generate and test numerous hypotheses regarding transcriptional and epigenetic regulations. However, it remains to be still challenging to conduct ChIP on an “epigenome” level, since ensuring an antibody of high specificity is often laborious and time-consuming. Another important concern with ChIP scalability is the maximum range of target regions that can be investigated by a single assay. For instance, a typical experiment of ChIP coupled with qPCR (i.e.,,ChIP-qPCR) is designed to measure the enrichment levels of a DNA binding protein at a handful of sites (e.g., gene promoters). However, in general, even a single epigenetic event in the cell pervasively occurs over a wide range of genomic regions, often involving thousands of genes and their associated regulatory elements. Thus, it becomes more important to have an ability to run the assay on a genome-wide scale for having a more balanced and unbiased perspective on the underlying mechanisms. Coupled with genomic profiling technologies such as tiling arrays or next generation sequencing (NGS), ChIP can be extended over the whole genome. In the following sections, we will introduce two major methods coupled with ChIP that enable epigenome-scale research of histone marks and transcription factors.

**ChIP-chip:** ChIP-chip is based on the combination of ChIP and a genomic tiling array technology (i.e.,, chip), in which DNA sequences extracted after ChIP hybridize with probes that are designed to cover the whole genome or specific regions of interest such as promoter[[75](#_ENREF_75),[76](#_ENREF_76)]. Due to bias in microarray hybridization, a control experiment using chromatin input or DNA from non-specific Immunoprecipitation (IP) (e.g., IP against immunoglobulin G) is often recommended. Most algorithms for ChIP-chip are designed to compute the normalized ratio between the hybridizations of ChIP and control after removing random and/or systemic noise. Then, they call binding sites as those significantly enriched in ChIP over control[[77-79](#_ENREF_77)]. Since its emergence in the early and mid 2000s, ChIP-chip has been widely adopted in many transcriptional and epigenetic regulation studies, assisting scientists to more understand the role of each histone mark in physiological and pathological processes[[80-83](#_ENREF_80)]. However, the utility of ChIP-chip is heavily restricted by a tiling array probe design, which determines the resolution of the measurement (i.e.,intervals between adjacent probes) and the regions that can be explored (e.g.,omission of repetitive sequence areas). These weaknesses of ChIP-chip have accelerated the major platform shift to NGS.

**ChIP-Seq:** In ChIP-Seq, the extracted DNA sequences are directly sequenced using a NGS technology instead of being hybridized onto tiling arrays. NGS refers to sequencing technologies that newly emerged since the mid 2000s as an alternative to the traditional automated Sanger sequencing. NGS is characterized as massive parallel sequencing of template DNA or RNA (cDNA) molecules by a relatively short length ranging over 50-400 bp[[84](#_ENREF_84)]. One advantage of ChIP-Seq over ChIP-chip is that ChIP-Seq does not require any predefined array design, which allows a more unbiased assay at a much higher resolution (100-1000 bp in ChIP-chip *vs* 10-100 bp in ChIP-Seq). Since NGS generally produces a notoriously large amount of data than array-based methods, more powerful bioinformatics support is essential for data processing and analysis[[85](#_ENREF_85),[86](#_ENREF_86)]. Bioinformatics analysis for ChIP-Seq in epigenetic research includes the pre-processing for sequence data such as quality control and read mapping, the identification of candidate sites enriched by the target histone mark, and further down-stream analysis for revealing biological implications of the observations from the precedent steps [[85](#_ENREF_85), [86](#_ENREF_86)].

In cancer studies, the down-stream analysis is focused on finding the most associated genes or regulatory elements (e.g., promoters or enhancers) with the histone mark of interest and investigating how these genes and regulatory elements can be understood in the context of biological pathways. Since Barski *et al*[[87](#_ENREF_87)] and Wang *et al*[[88](#_ENREF_88)] studies on 19 histone methylations and 18 histone acetylations using the human CD4+ T cell, many studies have been done to understand the biological implications of histone marks in normal conditions[[80-82](#_ENREF_80)]. However, due to the plasticity of epigenome and heterogeneity of cancer, cancer epigenetics of examining histone modifications on a genome scale still remains in its beginning stage. For this reason, most of currently on-going efforts in cancer epigenetics still largely target DNA methylation (e.g., The Cancer Genome Atlas, http://cancergenome.nih.gov/)[[89](#_ENREF_89)]. Therefore, it will be a long-term goal to accumulate the knowledge on cancer epigenetics from histone modifications and use it for cancer studies, which will require a great amount of public and private investments. Another interesting research direction is an attempt to comprehend how genetic variations lead to epigenetic changes in cancer. In 2011, several studies have been published about the possibility of multiple chromatin remodelers and histone enzymes as potential oncogenes or tumor suppressor genes[[90-93](#_ENREF_90)]. These studies suggest that the disruption of chromatin remodelers and histone enzymes due to driving somatic mutations in their coding regions may cause aberrant epigenetic changes, which eventually lead to cancer development or evolution in at least several cancer indications. Such approach is particularly interesting because it may be able to provide a genuine perspective on the target histone mark by observing somatic mutations in several key chromatin remodelers and histone enzymes.

In conclusion, a number of aberrant epigenetic modifications have been found in cancer cells, and diet and dietary factors play an important role to prevent cancer as well as to stimulate carcinogenesis. The use of epigenetic technology offers significant advantages to study the epigenetic mechanisms of cancer development and progression. Also, the newly developed technologies for epigenetic study expand the scope of nutrition study in the field of cancer research by helping monitor and pin down specific epigenetic pathways in diet-related cancers.

**REFERENCES**

1 **Egger G**, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004; **429**: 457-463 [PMID: 15164071 DOI: 10.1038/nature02625]

2 **Trosko JE**, Upham BL. The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. *Mutagenesis* 2005; **20**: 81-92 [PMID: 15784692]

3 **Jones PA**, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415-428 [PMID: 12042769 DOI: 10.1038/nrg816]

4 **Feinberg AP**, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer* 2004; **4**: 143-153 [PMID: 14732866 DOI: 10.1038/nrc1279]

5 **Lund AH**, van Lohuizen M. Epigenetics and cancer. *Genes Dev* 2004; **18**: 2315-2335 [PMID: 15466484]

6 **Hake SB**, Xiao A, Allis CD. Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br J Cancer* 2004; **90**: 761-769 [PMID: 14970850 DOI: 10.1038/sj.bjc.6601575]

7 **Esteller M**. Aberrant DNA methylation as a cancer-inducing mechanism. *Annu Rev Pharmacol Toxicol* 2005; **45**: 629-656 [PMID: 15822191 DOI: 10.1146/annurev.pharmtox.45.120403.095832]

8 **Esteller M**. Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. *Br J Cancer* 2006; **94**: 179-183 [PMID: 16404435]

9 **Fraga MF**, Esteller M. Towards the human cancer epigenome: a first draft of histone modifications. *Cell Cycle* 2005; **4**: 1377-1381 [PMID: 16205112]

10 **Luger K**, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 1997; **389**: 251-260 [PMID: 9305837 DOI: 10.1038/38444]

11 **Strahl BD**, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**: 41-45 [PMID: 10638745 DOI: 10.1038/47412]

12 **Fischle W**, Wang Y, Allis CD. Binary switches and modification cassettes in histone biology and beyond. *Nature* 2003; **425**: 475-479 [PMID: 14523437 DOI: 10.1038/nature02017]

13 **Seligson DB**, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdistani SK. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005; **435**: 1262-1266 [PMID: 15988529 DOI: 10.1038/nature03672]

14 **Tzao C**, Tung HJ, Jin JS, Sun GH, Hsu HS, Chen BH, Yu CP, Lee SC. Prognostic significance of global histone modifications in resected squamous cell carcinoma of the esophagus. *Mod Pathol* 2009; **22**: 252-260 [PMID: 18953329 DOI: 10.1038/modpathol.2008.172]

15 **Pogribny IP**, Ross SA, Tryndyak VP, Pogribna M, Poirier LA, Karpinets TV. 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 [PMID: 16497704 DOI: 10.1093/carcin/bgi364]

16 **Ghoshal AK**, Farber E. The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens. *Carcinogenesis* 1984; **5**: 1367-1370 [PMID: 6488458 DOI: 10.1093/carcin/5.10.1367]

17 **Newberne PM**. Lipotropic factors and oncogenesis. *Adv Exp Med Biol* 1986; **206**: 223-251 [PMID: 3035898]

18 **Poirier LA**. Methyl group deficiency in hepatocarcinogenesis. *Drug Metab Rev* 1994; **26**: 185-199 [PMID: 8082564 DOI: 10.3109/03602539409029790]

19 **Denda A**, Kitayama W, Kishida H, Murata N, Tsutsumi M, Tsujiuchi T, Nakae D, Konishi Y. Development of hepatocellular adenomas and carcinomas associated with fibrosis in C57BL/6J male mice given a choline-deficient, L-amino acid-defined diet. *Jpn J Cancer Res* 2002; **93**: 125-132 [PMID: 11856475 DOI: 10.1111/j.1349-7006.2002.tb01250.x]

20 **Wainfan E**, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. *Cancer Res* 1992; **52**: 2071s-2077s [PMID: 1544143]

21 **Christman JK**, Sheikhnejad G, Dizik M, Abileah S, Wainfan E. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis* 1993; **14**: 551-557 [PMID: 8472313 DOI: 10.1093/carcin/14.4.551]

22 **Pogribny IP**, James SJ, 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 [PMID: 15063136 DOI: 10.1016/j.mrfmmm.2003.12.014]

23 **Pogribny IP**, Ross SA, Wise C, Pogribna M, Jones EA, Tryndyak VP, James SJ, Dragan YP, Poirier LA. Irreversible global DNA hypomethylation as a key step in hepatocarcinogenesis induced by dietary methyl deficiency. *Mutat Res* 2006; **593**: 80-87 [PMID: 16144704 DOI: 10.1016/j.mrfmmm.2005.06.028]

24 **Ghoshal K**, Li X, Datta J, Bai S, Pogribny I, Pogribny M, Huang Y, Young D, Jacob ST. 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 [PMID: 16702315]

25 **Christman JK**, Weich N, Schoenbrun B, Schneiderman N, Acs G. Hypomethylation of DNA during differentiation of Friend erythroleukemia cells. *J Cell Biol* 1980; **86**: 366-370 [PMID: 6931107 DOI: 10.1083/jcb.86.2.366]

26 **Balaghi M**, Wagner C. DNA methylation in folate deficiency: use of CpG methylase. *Biochem Biophys Res Commun* 1993; **193**: 1184-1190 [PMID: 8323540 DOI: 10.1006/bbrc.1993.1750]

27 **Kim YI**, Giuliano A, Hatch KD, Schneider A, Nour MA, Dallal GE, Selhub J, Mason JB. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. *Cancer* 1994; **74**: 893-899 [[DOI: 10.1002/1097-0142(19940801)74:3<893::AID-CNCR2820740316>3.0.CO;2-B](http://dx.doi.org/10.1002/1097-0142%2819940801%2974%3A3%3C893%3A%3AAID-CNCR2820740316%3E3.0.CO;2-B)]

28 **Kim YI**, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, Mason JB. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. *Am J Clin Nutr* 1997; **65**: 46-52 [PMID: 8988912]

29 **Pogribny I**, Yi P, James SJ. A sensitive new method for rapid detection of abnormal methylation patterns in global DNA and within CpG islands. *Biochem Biophys Res Commun* 1999; **262**: 624-628 [PMID: 10471374 DOI: 10.1006/bbrc.1999.1187]

30 **Pogribny IP**, Shpyleva SI, Muskhelishvili L, Bagnyukova TV, James SJ, Beland FA. 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 [PMID: 19442675 DOI: 10.1016/j.mrfmmm.2009.05.003]

31 **Oakeley EJ**. DNA methylation analysis: a review of current methodologies. *Pharmacol Ther* 1999; **84**: 389-400 [PMID: 10665836 DOI: 10.1016/S0163-7258(99)00043-1]

32 **Friso S**, Choi SW, Dolnikowski GG, Selhub J. A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. *Anal Chem* 2002; **74**: 4526-4531 [PMID: 12236365 DOI: 10.1021/ac020050h]

33 **Kuo KC**, McCune RA, Gehrke CW, Midgett R, Ehrlich M. Quantitative reversed-phase high performance liquid chromatographic determination of major and modified deoxyribonucleosides in DNA. *Nucleic Acids Res* 1980; **8**: 4763-4776 [PMID: 7003544 DOI: 10.1093/nar/8.20.4763]

34 **Christman JK**. Separation of major and minor deoxyribonucleoside monophosphates by reverse-phase high-performance liquid chromatography: a simple method applicable to quantitation of methylated nucleotides in DNA. *Anal Biochem* 1982; **119**: 38-48 [PMID: 7072940 DOI: 10.1016/0003-2697(82)90662-5]

35 **Kaur H**, Halliwell B. Measurement of oxidized and methylated DNA bases by HPLC with electrochemical detection. *Biochem J* 1996; **318 (Pt 1)**: 21-23 [PMID: 8761446]

36 **Friso S**, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci USA* 2002; **99**: 5606-5611 [PMID: 11929966 DOI: 10.1073/pnas.062066299]

37 **Choi SW**, Friso S, Ghandour H, Bagley PJ, Selhub J, Mason JB. Vitamin B-12 deficiency induces anomalies of base substitution and methylation in the DNA of rat colonic epithelium. *J Nutr* 2004; **134**: 750-755 [PMID: 15051821]

38 **Friso S**, Girelli D, Trabetti E, Olivieri O, Guarini P, Pignatti PF, Corrocher R, Choi SW. The MTHFR 1298A& gt; C polymorphism and genomic DNA methylation in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 938-943 [PMID: 15824167 DOI: 10.1158/1055-9965.EPI-04-0601]

39 **Keyes MK**, Jang H, Mason JB, Liu Z, Crott JW, Smith DE, Friso S, Choi SW. Older age and dietary folate are determinants of genomic and p16-specific DNA methylation in mouse colon. *J Nutr* 2007; **137**: 1713-1717 [PMID: 17585020]

40 **Lim U**, Flood A, Choi SW, Albanes D, Cross AJ, Schatzkin A, Sinha R, Katki HA, Cash B, Schoenfeld P, Stolzenberg-Solomon R. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. *Gastroenterology* 2008; **134**: 47-55 [PMID: 18166347 DOI: 10.1053/j.gastro.2007.10.013]

41 **Bird AP**. Use of restriction enzymes to study eukaryotic DNA methylation: II. The symmetry of methylated sites supports semi-conservative copying of the methylation pattern. *J Mol Biol* 1978; **118**: 49-60 [PMID: 625057 DOI: 10.1016/0022-2836(78)90243-7]

42 **Waalwijk C**, Flavell RA. MspI, an isoschizomer of hpaII which cleaves both unmethylated and methylated hpaII sites. *Nucleic Acids Res* 1978; **5**: 3231-3236 [PMID: 704354 DOI: 10.1093/nar/5.9.3231]

43 **Razin A**, Riggs AD. DNA methylation and gene function. *Science* 1980; **210**: 604-610 [PMID: 6254144 DOI: 10.1126/science.6254144]

44 **Feinberg AP**, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* 1983; **301**: 89-92 [PMID: 6185846 DOI: 10.1038/301089a0]

45 **Goelz SE**, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* 1985; **228**: 187-190 [PMID: 2579435 DOI: 10.1126/science.2579435]

46 **Singer-Sam J**, LeBon JM, Tanguay RL, Riggs AD. A quantitative HpaII-PCR assay to measure methylation of DNA from a small number of cells. *Nucleic Acids Res* 1990; **18**: 687 [PMID: 1689825 DOI: 10.1093/nar/18.3.687]

47 **Frommer M**, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, Molloy PL, Paul CL. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. *Proc Natl Acad Sci U S A* 1992; **89**: 1827-1831 [PMID: 1542678 DOI: 10.1073/pnas.89.5.1827]

48 **Eckhardt F**, Lewin J, Cortese R, Rakyan VK, Attwood J, Burger M, Burton J, Cox TV, Davies R, Down TA, Haefliger C, Horton R, Howe K, Jackson DK, Kunde J, Koenig C, Liddle J, Niblett D, Otto T, Pettett R, Seemann S, Thompson C, West T, Rogers J, Olek A, Berlin K, Beck S. DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat Genet* 2006; **38**: 1378-1385 [PMID: 17072317 DOI: 10.1038/ng1909]

49 **Herman JG**, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A* 1996; **93**: 9821-9826 [PMID: 8790415 DOI: 10.1073/pnas.93.18.9821]

50 **Xiong Z**, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. *Nucleic Acids Res* 1997; **25**: 2532-2534 [PMID: 9171110 DOI: 10.1093/nar/25.12.2532]

51 **Eads CA**, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, Danenberg PV, Laird PW. MethyLight: a high-throughput assay to measure DNA methylation. *Nucleic Acids Res* 2000; **28**: E32 [PMID: 10734209 DOI: 10.1093/nar/28.8.e32]

52 **Dupont JM**, Tost J, Jammes H, Gut IG. De novo quantitative bisulfite sequencing using the pyrosequencing technology. *Anal Biochem* 2004; **333**: 119-127 [PMID: 15351288 DOI: 10.1016/j.ab.2004.05.007]

53 **Gitan RS**, Shi H, Chen CM, Yan PS, Huang TH. Methylation-specific oligonucleotide microarray: a new potential for high-throughput methylation analysis. *Genome Res* 2002; **12**: 158-164 [PMID: 11779841 DOI: 10.1101/gr.202801]

54 **Weber M**, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schübeler D. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet* 2005; **37**: 853-862 [PMID: 16007088 DOI: 10.1038/ng1598]

55 **Khulan B**, Thompson RF, Ye K, Fazzari MJ, Suzuki M, Stasiek E, Figueroa ME, Glass JL, Chen Q, Montagna C, Hatchwell E, Selzer RR, Richmond TA, Green RD, Melnick A, Greally JM. Comparative isoschizomer profiling of cytosine methylation: the HELP assay. *Genome Res* 2006; **16**: 1046-1055 [PMID: 16809668 DOI: 10.1101/gr.5273806]

56 **Allinen M**, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* 2004; **6**: 17-32 [PMID: 15261139 DOI: 10.1016/j.ccr.2004.06.010]

57 **Bingham SA**. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proc Nutr Soc* 1990; **49**: 153-171 [PMID: 2172992 DOI: 10.1079/PNS19900021]

58 **Murphy N**, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, Dahm CC, Overvad K, Olsen A, Tjønneland A, Clavel-Chapelon F, Boutron-Ruault MC, Racine A, Kaaks R, Teucher B, Boeing H, Bergmann MM, Trichopoulou A, Trichopoulos D, Lagiou P, Palli D, Pala V, Panico S, Tumino R, Vineis P, Siersema P, van Duijnhoven F, Peeters PH, Hjartaker A, Engeset D, González CA, Sánchez MJ, Dorronsoro M, Navarro C, Ardanaz E, Quirós JR, Sonestedt E, Ericson U, Nilsson L, Palmqvist R, Khaw KT, Wareham N, Key TJ, Crowe FL, Fedirko V, Wark PA, Chuang SC, Riboli E. Dietary fibre intake and risks of cancers of the colon and rectum in the European prospective investigation into cancer and nutrition (EPIC). *PLoS One* 2012; **7**: e39361 [PMID: 22761771 DOI: 10.1371/journal.pone.0039361]

59 **Topping DL**, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 2001; **81**: 1031-1064 [PMID: 11427691]

60 **McIntyre A**, Gibson PR, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut* 1993; **34**: 386-391 [PMID: 8386131 DOI: 10.1136/gut.34.3.386]

61 **Gobbi G**, Di Marcantonio D, Micheloni C, Carubbi C, Galli D, Vaccarezza M, Bucci G, Vitale M, Mirandola P. TRAIL up-regulation must be accompanied by a reciprocal PKCε down-regulation during differentiation of colonic epithelial cell: implications for colorectal cancer cell differentiation. *J Cell Physiol* 2012; **227**: 630-638 [PMID: 21465464 DOI: 10.1002/jcp.22765]

62 **Gope R**, Gope ML. Effect of sodium butyrate on the expression of retinoblastoma (RB1) and P53 gene and phosphorylation of retinoblastoma protein in human colon tumor cell line HT29. *Cell Mol Biol (Noisy-le-grand)* 1993; **39**: 589-597 [PMID: 8220069]

63 **Nakano K**, Mizuno T, Sowa Y, Orita T, Yoshino T, Okuyama Y, Fujita T, Ohtani-Fujita N, Matsukawa Y, Tokino T, Yamagishi H, Oka T, Nomura H, Sakai T. Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. *J Biol Chem* 1997; **272**: 22199-22206 [PMID: 9268365 DOI: 10.1074/jbc.272.35.22199]

64 **Fang JY**, Chen YX, Lu J, Lu R, Yang L, Zhu HY, Gu WQ, Lu LG. Epigenetic modification regulates both expression of tumor-associated genes and cell cycle progressing in human colon cancer cell lines: Colo-320 and SW1116. *Cell Res* 2004; **14**: 217-226 [PMID: 15225415 DOI: 10.1038/sj.cr.7290222]

65 **Janson W**, Brandner G, Siegel J. Butyrate modulates DNA-damage-induced p53 response by induction of p53-independent differentiation and apoptosis. *Oncogene* 1997; **15**: 1395-1406 [PMID: 9333015 DOI: 10.1038/sj.onc.1201304]

66 **Terui T**, Murakami K, Takimoto R, Takahashi M, Takada K, Murakami T, Minami S, Matsunaga T, Takayama T, Kato J, Niitsu Y. Induction of PIG3 and NOXA through acetylation of p53 at 320 and 373 lysine residues as a mechanism for apoptotic cell death by histone deacetylase inhibitors. *Cancer Res* 2003; **63**: 8948-8954 [PMID: 14695212]

67 **Bernhard D**, Ausserlechner MJ, Tonko M, Löffler M, Hartmann BL, Csordas A, Kofler R. Apoptosis induced by the histone deacetylase inhibitor sodium butyrate in human leukemic lymphoblasts. *FASEB J* 1999; **13**: 1991-2001 [PMID: 10544182]

68 **Kuwajima A**, Iwashita J, Murata J, Abe T. The histone deacetylase inhibitor butyrate inhibits melanoma cell invasion of Matrigel. *Anticancer Res* 2007; **27**: 4163-4169 [PMID: 18225587]

69 **Van Lint C**, Emiliani S, Verdin E. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr* 1996; **5**: 245-253 [PMID: 8723390]

70 **Mariadason JM**, Corner GA, Augenlicht LH. Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. *Cancer Res* 2000; **60**: 4561-4572 [PMID: 10969808]

71 **Davie JR**. Inhibition of histone deacetylase activity by butyrate. *J Nutr* 2003; **133**: 2485S-2493S [PMID: 12840228]

72 **Jackson V**, Shires A, Chalkley R, Granner DK. Studies on highly metabolically active acetylation and phosphorylation of histones. *J Biol Chem* 1975; **250**: 4856-4863 [PMID: 168194]

73 **Boffa LC**, Vidali G, Mann RS, Allfrey VG. Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. *J Biol Chem* 1978; **253**: 3364-3366 [PMID: 649576]

74 **Collas P**. The current state of chromatin immunoprecipitation. *Mol Biotechnol* 2010; **45**: 87-100 [PMID: 20077036 DOI: 10.1007/s12033-009-9239-8]

75 **de Magalhaes JP**, Finch CE, Janssens G. Next-generation sequencing in aging research: emerging applications, problems, pitfalls and possible solutions. *Ageing research reviews* 2010; **9**: 315-323 [DOI: 10.1016/j.arr.2009.10.006]

76 **Ho JWK**, Bishop E, Karchenko PV, Negre N, White KP, Park PJ. ChIP-chip versus ChIP-seq: lessons for experimental design and data analysis. *BMC Genomics* 2011; **12**: 134 [DOI: 10.1186/1471-2164-12-134]

77 **Johnson WE**, Li W, Meyer CA, Gottardo R, Carroll JS, Brown M, Liu XS. Model-based analysis of tiling-arrays for ChIP-chip. *Proc Natl Acad Sci USA* 2006; **103**: 12457-12462 [PMID: 16895995 DOI: 10.1073/pnas.0601180103]

78 **Song J**, Johnson W, Zhu X, Zhang X, Li W, Manrai A, Liu J, Chen R, Liu X. Model-based analysis of two-color arrays (MA2C). *Genome Biol* 2007; **8**: R178 [DOI: 10.1186/gb-2007-8-8-r178]

79 **Ji H**, Jiang H, Ma W, Johnson DS, Myers RM, Wong WH. An integrated software system for analyzing ChIP-chip and ChIP-seq data. *Nature Biotechnology* 2008; **26**: 1293-1300 [DOI: 10.1038/nbt.1505]

80 **Consortium EP**. The ENCODE (ENCyclopedia Of DNA Elements) Project. *Science* 2004; **306**(5696): 636-640 [DOI: 10.1126/science.1105136]

81 **Liu T**, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre I, Cheung M-S, Ercan S, Ikegami K, Jensen M, Kolasinska-Zwierz P, Rosenbaum H, Shin H, Taing S, Takasaki T, Iniguez AL, Desai A, Dernburg AF, Kimura H, Lieb JD, Ahringer J, Strome S, Liu XS. Broad chromosomal domains of histone modification patterns in C. elegans. *Genome Research* 2011; **21**: 227-236 [DOI: 10.1101/gr.115519.110]

82 **Consortium M**, Roy S, Ernst J, Kharchenko PV, Kheradpour P, Negre N, Eaton ML, Landolin JM, Bristow CA, Ma L, Lin MF, Washietl S, Arshinoff BI, Ay F, Meyer PE, Robine N, Washington NL, Di Stefano L, Berezikov E, Brown CD, Candeias R, Carlson JW, Carr A, Jungreis I, Marbach D, Sealfon R, Tolstorukov MY, Will S, Alekseyenko AA, Artieri C, Booth BW, Brooks AN, Dai Q, Davis CA, Duff MO, Feng X, Gorchakov AA, Gu T, Henikoff JG, Kapranov P, Li R, MacAlpine HK, Malone J, Minoda A, Nordman J, Okamura K, Perry M, Powell SK, Riddle NC, Sakai A, Samsonova A, Sandler JE, Schwartz YB, Sher N, Spokony R, Sturgill D, van Baren M, Wan KH, Yang L, Yu C, Feingold E, Good P, Guyer M, Lowdon R, Ahmad K, Andrews J, Berger B, Brenner SE, Brent MR, Cherbas L, Elgin SCR, Gingeras TR, Grossman R, Hoskins RA, Kaufman TC, Kent W, Kuroda MI, Orr-Weaver T, Perrimon N, Pirrotta V, Posakony JW, Ren B, Russell S, Cherbas P, Graveley BR, Lewis S, Micklem G, Oliver B, Park PJ, Celniker SE, Henikoff S, Karpen GH, Lai EC, Macalpine DM, Stein LD, White KP, Kellis M. Identification of functional elements and regulatory circuits by Drosophila modENCODE. *Science* 2010; **330**(6012): 1787-1797 [DOI: 10.1126/science.1198374]

83 **Bernstein BE**, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, Kellis M, Marra MA, Beaudet AL, Ecker JR, Farnham PJ, Hirst M, Lander ES, Mikkelsen TS, Thomson JA. The NIH Roadmap Epigenomics Mapping Consortium. *Nature Biotechnology* 2010; **28**: 1045-1048 [DOI: 10.1038/nbt1010-1045]

84 **Metzker ML**. Sequencing technologies - the next generation. *Nature Reviews Genetics* 2010; **11**(1): 31-46 [DOI: 10.1038/nrg2626]

85 **Kharchenko PV**, Tolstorukov MY, Park PJ. Design and analysis of ChIP-seq experiments for DNA-binding proteins. *Nature Biotechnology* 2008; **26**(12): 1351-1359 [DOI: 10.1038/nbt.1508]

86 **Park PJ**. ChIP-seq: advantages and challenges of a maturing technology. *Nature Reviews Genetics* 2009; **10**(10): 669-680 [DOI: 10.1038/nrg2641]

87 **Barski A**, Cuddapah S, Cui K, Roh T-Y, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell* 2007; **129**(4): 823-837 [DOI: 10.1016/j.cell.2007.05.009]

88 **Wang Z**, Zang C, Rosenfeld J, Schones D, Barski A. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nature Genetics* 2008 [DOI: 10.1038/ng.154]

89 Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008; **455**: 1061-1068 [PMID: 18772890 DOI: 10.1038/nature07385]

90 **Morin RD**, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M, Jackman S, Krzywinski M, Scott DW, Trinh DL, Tamura-Wells J, Li S, Firme MR, Rogic S, Griffith M, Chan S, Yakovenko O, Meyer IM, Zhao EY, Smailus D, Moksa M, Chittaranjan S, Rimsza L, Brooks-Wilson A, Spinelli JJ, Ben-Neriah S, Meissner B, Woolcock B, Boyle M, McDonald H, Tam A, Zhao Y, Delaney A, Zeng T, Tse K, Butterfield Y, Birol I, Holt R, Schein J, Horsman DE, Moore R, Jones SJ, Connors JM, Hirst M, Gascoyne RD, Marra MA. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature* 2011; **476**: 298-303 [PMID: 21796119 DOI: 10.1038/nature10351]

91 **Pasqualucci L**, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, Wells VA, Grunn A, Messina M, Elliot O, Chan J, Bhagat G, Chadburn A, Gaidano G, Mullighan CG, Rabadan R, Dalla-Favera R. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat Genet* 2011; **43**: 830-837 [PMID: 21804550 DOI: 10.1038/ng.892]

92 **Gui Y**, Guo G, Huang Y, Hu X, Tang A, Gao S, Wu R, Chen C, Li X, Zhou L, He M, Li Z, Sun X, Jia W, Chen J, Yang S, Zhou F, Zhao X, Wan S, Ye R, Liang C, Liu Z, Huang P, Liu C, Jiang H, Wang Y, Zheng H, Sun L, Liu X, Jiang Z, Feng D, Chen J, Wu S, Zou J, Zhang Z, Yang R, Zhao J, Xu C, Yin W, Guan Z, Ye J, Zhang H, Li J, Kristiansen K, Nickerson ML, Theodorescu D, Li Y, Zhang X, Li S, Wang J, Yang H, Wang J, Cai Z. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet* 2011; **43**: 875-878 [PMID: 21822268 DOI: 10.1038/ng.907]

93 **Li M**, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, Pawlik TM, Daniel HD, Kannangai R, Offerhaus GJ, Velculescu VE, Wang L, Zhou S, Vogelstein B, Hruban RH, Papadopoulos N, Cai J, Torbenson MS, Kinzler KW. Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. *Nat Genet* 2011; **43**: 828-829 [PMID: 21822264 DOI: 10.1038/ng.903]

94 **Pogribny IP**, James SJ. 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 [PMID: 12359353]

95 **Tsujiuchi T**, Shimizu K, Itsuzaki Y, Onishi M, Sugata E, Fujii H, Honoki K. 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 [PMID: 17295234 DOI: 10.1002/mc.20268]

96 **Pogribny IP**, Tryndyak VP, Bagnyukova TV, Melnyk S, Montgomery B, Ross SA, Latendresse JR, Rusyn I, Beland FA. Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. *J Hepatol* 2009; **51**: 176-186 [PMID: 19450891 DOI: 10.1016/j.jhep.2009.03.021]

97 **Tryndyak VP**, Han T, Muskhelishvili L, Fuscoe JC, Ross SA, Beland FA, Pogribny IP. 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 [PMID: 20938992 DOI: 10.1002/mnfr.201000300]

98 **Demary K**, Wong L, Spanjaard RA. Effects of retinoic acid and sodium butyrate on gene expression, histone acetylation and inhibition of proliferation of melanoma cells. *Cancer Lett* 2001; **163**: 103-107 [PMID: 11163113]

99 **Kida Y**, Shimizu T, Kuwano K. Sodium butyrate up-regulates cathelicidin gene expression via activator protein-1 and histone acetylation at the promoter region in a human lung epithelial cell line, EBC-1. *Mol Immunol* 2006; **43**: 1972-1981 [PMID: 16423398 DOI: 10.1016/j.molimm.2005.11.014]

100 **Rada-Iglesias A**, Enroth S, Ameur A, Koch CM, Clelland GK, Respuela-Alonso P, Wilcox S, Dovey OM, Ellis PD, Langford CF, Dunham I, Komorowski J, Wadelius C. Butyrate mediates decrease of histone acetylation centered on transcription start sites and down-regulation of associated genes. *Genome Res* 2007; **17**: 708-719 [PMID: 17567991 DOI: 10.1101/gr.5540007]

**P-Reviewers** Prasad KK, Lakatos PL **S-Editor** Huang XZ  **L-Editor E-Editor**

**Figure 1 Effect of methyl-deficiency on biological methylation pathway.** S-AdoMet: S-adenosylmethionine; S-AdoHcy: S-adensylhomocysteine; DNMT: DNA methyltransferase; SAHH: S-adenosylhomocysteine hydrolase; MS: Methionine synthase; THF: Tetrahydrofolate; MTHFR: Methylenetetrahydrofolate reductase.

**Table 1 DNA methylation analyses used in methyl-deficient model of hepatocarcinogenesis in rodents**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dietary component | Model | Observations | Methylation assay | Ref. |
| Amino acid-defined diet lacking choline, methionine, folic acid and vitamin B12  | Rat | Depletion of SAdoMet and DNA hypomethylation  | Liver DNA methyltransferase activity assay with labeled SAdoMet  | [[20](#_ENREF_20)] |
| Amino acid-defined diet lacking choline, methionine, folic acid and vitamin B12 | Rat | Hypomethylation of CCGG site of c-myc, c-fos and c-Ha-ras | Enzyme digestion by HpaII/MspI | [[21](#_ENREF_21)] |
| Diet low in methionine lacking in choline and folic acid | Rat | Hypermethylation of p16INK4A | MS-PCR  | [[94](#_ENREF_94)] |
| Diet low in methionine lacking in choline and folic acid | Rat | Decrease in the total percent of methylated CCGG sites in DNA | HpaII/MspI-based cytosine extension assay | [[22](#_ENREF_22)] |
| Diet low in methionine lacking in choline and folic acid | Rat | Depletion of S-AdoMet, decrease in S-AdoMet/S-AdoHcy and global DNA hypomethylation | HpaII/MspI-based cytosine extension assay | [[23](#_ENREF_23)] |
| Diet low in methionine lacking in choline and folic acid | Rat | Hypomethylation of ID element and LINE-1 in preneoplastic livers and liver tumorsDecrease in histone H4-Lys20 trimethylation and increase in histone H3-Lys9 trimethylationDecrease in histone H4-Lys20 trimethylation at the LINE-1 regulatory region | ID methylation by methylation-sensitive McrBC-PCR arrayLINE-1 methylation by COBRA-assay Global histone methylation by Western blottingLINE-1-associated histone methylation by ChIP | [[15](#_ENREF_15)] |
| Diet deficient in methionine lacking in choline and folic acid | Rat | Changes in the DNA methylation machinery | Indirect methods by DNA methyltransferases and Methyl CpG binding proteins | [[24](#_ENREF_24)] |
| Amino acid-defined diet lacking choline,  | Rat | Hypermethylation of upstream of E-cadherin and Cx26 | Bisulfite sequencing | [[95](#_ENREF_95)] |
| Diet deficient in methionine lacking in choline and folic acid | Rat | Global loss of DNA methylationHypermethylation of CpG islands  | Global DNA methylation by cytosine extension assay and [3H-methyl] incorporationCpG island methylation by [32P]dGTP incorporation | [[30](#_ENREF_30)] |
| Diet deficient in methionine lacking in choline and folic acid | Mouse | Global DNA hypomethylationSubstantial loss of repetitive sequences (LINE-1, SINES, IAP elements) cytosine methylationIncrease in histone H3-Lys9 trimethylation and decrease in histone H4-Lys20 trimethylation  | Global DNA methylation by cytosine extension assayMethylation-sensitive McrBC-qPCR assayGlobal histone modifications by Western blot | [[96](#_ENREF_96)] |
| Diet deficient in methionine lacking in choline and folic acid | Mouse | Detection of CpG island methylation profiles | MeDIP | [[97](#_ENREF_97)] |

MS-PCR: Methylation specific-PCR; ID: Identifier; LINE-1: Long interspersed nucleotide elements; ChIP: Chromatin immunoprecipitation; SINES: Short interspersed nuclear elements; IAP: Intracisternal A-particle; MeDIP: Methylated DNA immunoprecipitation.

**Table 2 A summary of selected evidence for effects of butyrate in histone modification and histone modification assays in cancer cell culture models**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Dietary component | Cell culture Model | Observations | Histone modification assay | Ref. |
| Sodium butyrate | SW620 human colon carcinoma cells | Increased global histone H4 acetylation | Western blot | [[70](#_ENREF_70)] |
| Sodium butyrate | A375 human melanoma and S91 mouse melanoma | Increased global histone H4 acetylation  | Western blot  | [[98](#_ENREF_98)] |
| Sodium butyrate |  Colo-320 human colon cancer cells | Increased acetylation of histone H3 and H4 within CDKN1A promoter site  | ChIP | [[64](#_ENREF_64)] |
| Sodium butyrate | EBC-1 human lung epithelial cells  | Increased histone H3 and H4 acetylation associated with promoter of cathelicidin | ChIP | [[99](#_ENREF_99)] |
| Sodium butyrate | HepG2 human hepatocarcinoma | Increased global histone H3 and H4 acetylationGenome-wide changes in acetylation of DNA-bound histones | Western blotChIP-chip (ChIP and microarray hybridization) | [[100](#_ENREF_100)] |

CDKN1A: Cyclin-dependent kinase inhibitor 1A; ChIP: Chromatin immunoprecipitation.