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Review article

Epigenetics and aging

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Abstract

Epigenetics is a mechanism precisely controlling gene expression by the change in chromatin structure, not accompanied by the change in the DNA sequence, in the processes of generation and differentiation. Representative cases of epigenetic changes are DNA methylation, histone acetylation and histone methylation. The metabolic pathway, including folic acid metabolism and methionine metabolism, is referred to as one-carbon metabolism. The nutrients constituting this metabolic pathway are methionine, folic acid and vitamin B_{12}. They have an effect on the epigenetic control mechanism and also affect gene expression. It is reported that the failure of these epigenetic mechanisms causes various disorders including some forms of cancer, life-style diseases and psychiatric diseases.

Recently, stem cell aging theory has been promoted, which states that the functions of stem cells are reduced with aging, and as a result the structuring and maintenance of the body tissues become difficult. It is pointed out that the epigenetic change of tissue stem cells over time plays an important role in the decline in the function of an individual caused by aging. It is quite likely that glycative stress affects epigenetic modification. In this research we are attempting to clarify the molecular mechanism of aging, focusing on the epigenetic change, using intestinal epithelial organoids structured by an organoid culture system, a new 3-dimentional stem cell culture method, and also the development of a new anti-aging intervention based on epigenome editing techniques.

***KEY WORDS*:** epigenetics, DNA methylation, histone modification, one-carbon metabolism, stem cell aging, anti-aging medicine

Control of Genetic Expression by Epigenetic Change

The epigenetic mechanism precisely controls gene expression by the change in chromatin structure, not accompanied by the change in the DNA sequence, and it is deeply involved in life phenomena such as differentiation, generation, imprinting and X-chromosome inactivation. Epigenetics is a change normally passed down even after cell division. If this mechanism is broken, it is reported to cause various disorders such as some forms of cancer, life-style diseases and psychiatric diseases [1-3]. Typical examples of epigenetic changes include DNA methylation histone acetylation and histone methylation.

DNA methylation is only one physiological modification of the genome of the vertebrate. It is a reaction where a methyl group is attached to the 5-position carbon atom of the cytosine of 2-nucleotide sequence (CpG) arranged in the order of cytosine (C) and guanine (G) from the 5'side of the DNA and it is catalyzed by DNA methyltransferase (DNMT) (***Fig. 1***) [2,3]. In many cases, the clusters of CpG sequence called "CpG island" are formed in the gene promoter region. It is known that, if the gene promoter region becomes methylated, the gene expression downstream is suppressed. Meanwhile, DNA demethylation can be broadly classified into passive demethylation generated depending upon DNA replication and active demethylation not depending upon DNA replication. It is considered that the TET (ten-eleven translocation) hydroxylation reaction plays an important role in the active demethylation [4]. TET hydroxylates 5-position methyl group of cytosine (5mC: 5-mehylcytosine) and converts it to 5hmC (5-hydroxymethyl cytosine) (***Fig. 1***). The converted 5hmC is converted to unmethylated cytosine through cell division and DNA base excision repair mechanism and as a result, it becomes demethylated. It is presumed that 5hmC plays a role as an intermediate in DNA demethylation pathways: however, it also possibly plays a new epigenetic regulatory role itself. As mentioned above, 5'mC is considered

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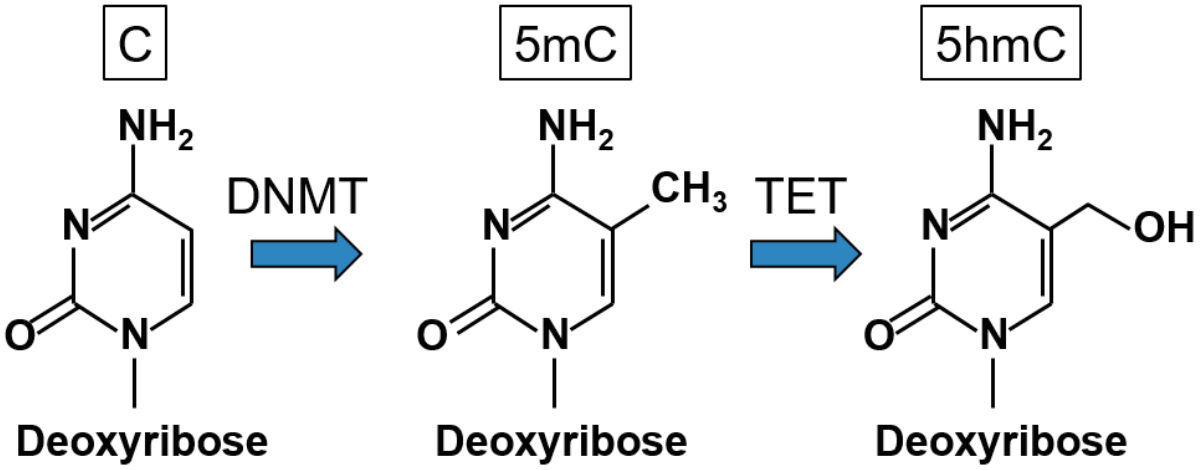

Fig. 1. **DNA methylation and demethylation.**

A methyl group is added to the 5-position of cytosine by DNA methyltransferase (DNMT) and it becomes 5-methyl-cytosine. TET hydroxylates the methyl group of the 5-position of cytosine (5mC:5-methyl cytosine) and converts it to 5hmC (5-hydroxymethyl cytosine). The converted 5hmC is converted to unmethylated cytosine through cell division and DNA base excision repair mechanism and as a result, becomes demethylated.

to be "the fifth base" and 5hmC is "the sixth base," and it is presumed that they play extremely important roles in many fields of the generation and progression of cancer, life-style diseases and neurological disorders. However, many unclear points regarding their biological roles remain.

In addition to DNA methylation, histone modification also plays an important role in gene expression. As shown in ***Fig. 2***, no DNA methylation is observed in the gene promoter region where generally active gene expression is observed, and histone is acetylated. Meanwhile, when histone H3 lysine 9 (H3K9) is methylated, it recruits DNMT and histone deacetylase (HDAC), and as result, DNA is methylated and histone is deacetylated. Chromatin structures are then agglutinated and gene expression is inactivated due to these modifications. Furthermore, as the controlling mechanism of gene expression is by an epigenetic mechanism, not through DNA methylation, the methylation of histone H3 lysine 27 (H3K27) by polycomb repressive complex (PRC) is reported (***Fig. 2***) [2,3].

As an important point, these epigenome changes are fundamentally reversible by utilizing drugs such as a DNA methylation inhibitor, histone deacetylase inhibitor and histone methyltransferase inhibitor. These drugs are attracting a lot of attention as molecular target drugs for diseases such as cancer for the next generation [5]. Actually, Azacytidine (Vidaza®), a DNA methylation inhibitor, and Vorinostat (Zolinza®), a histone deacetylase inhibitor, have been approved to be used for myelodysplastic syndromes (MDS) and cutaneous T-cell lymphoma, respectively, and their effects have been notable [6].

Metabolism and Epigenetics

Recently, one-carbon metabolism and the nutrients constituting it have drawn attention.

One-carbon metabolism refers to the metabolic pathway including folic acid metabolism and methionine metabolism. The nutrients constituting this metabolic pathway (methionine, folic acid and vitamin B_{12}) work on the epigenetic control mechanism, affecting gene expression and others, so that they are considered to play a very important role in diseases including cancer (***Fig. 3***). S-adenosylmethionine (SAM) is a metabolite regarded as best contributing to DNA methylation. SAM is synthesized by methionine adenosyltransferase (MAT) with methionine and ATP as substrates and it works as a methyl group donor for DNA methylation and histone methylation. The SAM that has donated its methyl group is converted to S-adenosylhomocysteine (SAH) (***Fig. 3***).

Folic acid deficiency disease decreases SAM in cells and inhibits the methylation of cytosine widely, and as a result, it causes the instability of the chromosome and the activation of the cancer gene, so that it is considered to increase cancer risk [7]. The epidemiologic studies so far suggest that there is an inverse correlation between the intake volume of folate and the risk of cancers such as colon cancer [8]. According to the cohort study, the NIH-AARP Diet and Health Study targeting more than 525,000 Americans aged from 50 to 71, those whose total intake amounts of folate were more than 900 μg/a day run a 30% lower risk of colon cancer compared with those of less than 200 μg/a day [9]. However, the details of the effects of folate against the onset of cancer have not been verified by research yet. It was also pointed out that a large intake volume of folic acid may possibly promote the onset of cancer and its progression [10, 11]. So far, it has been suggested that an appropriate intake of folate possibly lowers the risk of certain types of cancer. Meanwhile, a large volume of folate intake may adversely increase the risk of cancer. In order to fully clarify the roles of folate derived from food and included in supplements, in the development of colon cancer and other cancers, further research is required.

All cells performing living activities are using a glycolytic system as their energy source. Energy is produced within cells using glucose taken from the outside of cells.

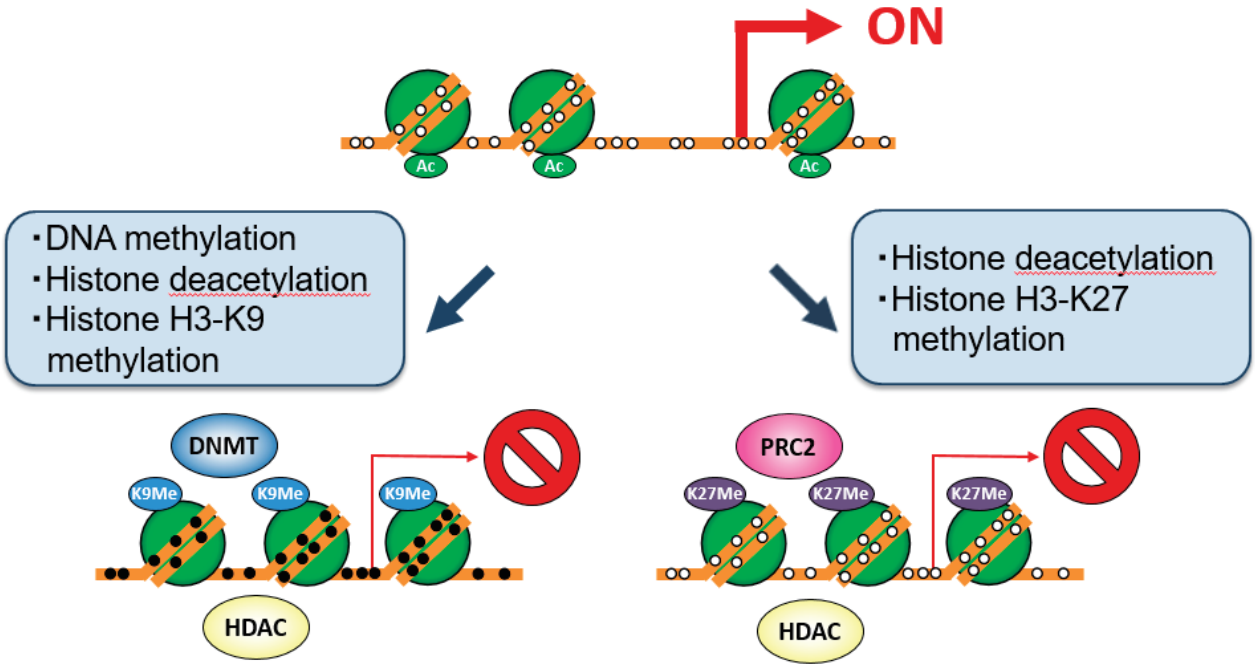

Fig. 2. **Control of gene expression by epigenetic change.**

When histone H3K9 is methylated, it recruits DNMT and HDAC, DNA is methylated and histone is deacetylated. Through these modifications, the chromatin structure is agglutinated and gene expression is deactivated. Meanwhile, when histone H3K27 is methylated by PRC2, it deactivates gene expression not through DNA methylation.

●: Methylated DNA, ○: Demethylated DNA, Ac: Acetylated histone, Me: Methylated histone.

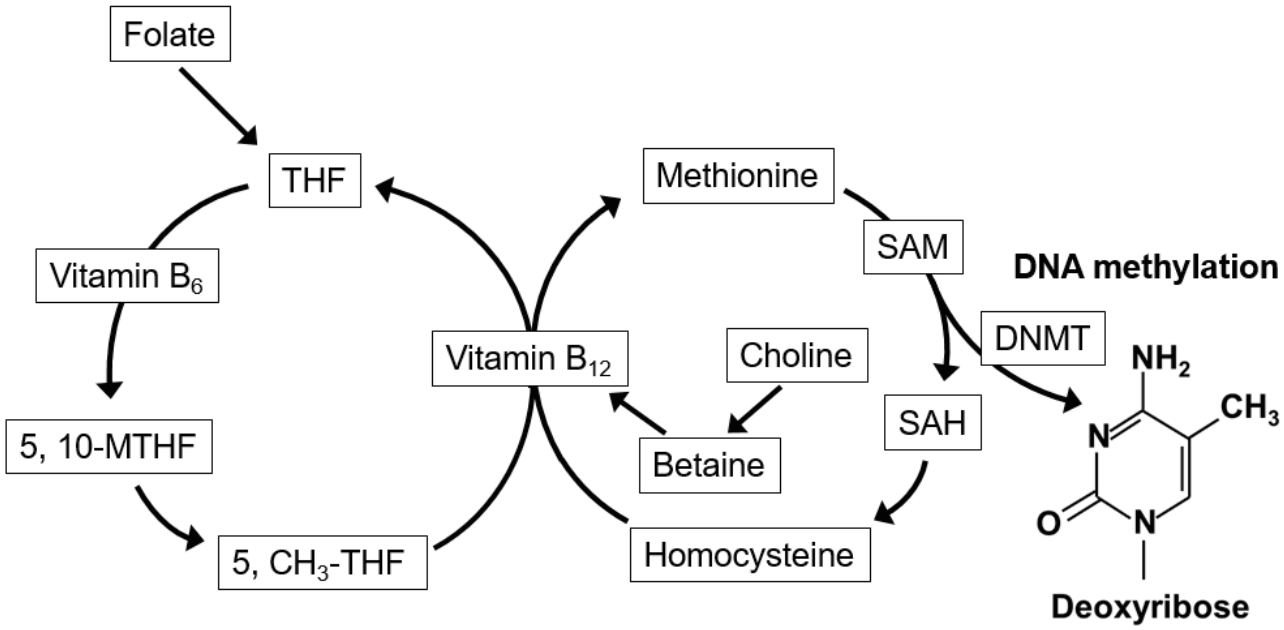

Fig. 3. **One-carbon metabolism and epigenetics.**

One-carbon metabolism is being examined as the mechanism combining nutrients and epigenetics. The nutrients constituting one-carbon metabolism bring about DNA methylation change. SAM of methionine metabolic pathway, in particular, is an important metabolic product playing a role of methyl group donor for DNA methylation and histone methylation.

THF: tetrahydrofolate; 5, 10-MTHF: 5, 10-methylene THF; 5, CH3-THF: 5, methyl-THF; SAM: S-adenosylmethionine; SAH: S-adenosylhomocysteine.

And, almost all biopolymers existing in a living body receive glycation (glycative reaction) caused by living activities. The effects of glycation on cells are called glycative stress. In the early days, the term glycation referred to the pathway where the carbonyl group of reducing sugars typified by glucose and the amino group of protein react non-enzymatically, which finally leads to the formation of advanced glycation end-products (AGEs). However, in the present day, the term is used in a broader range. As long as all proteins of a living body continue living activity, they possibly inevitably receive non-enzymatical glycative reactions. In the state of chronic hyperglycemia like diabetes, the glycative reactions accelerate everywhere in the living body, and it results in the accumulation of its final products, AGEs. In the present aging society, the more a body has aged, the more glycative reactions become involved in the onsets of age-related diseases including cancers. It is possible that the glycative reactions also affect epigenetics. Histone lysine carboxymethylation (HL-CM) formed under diabetic conditions, in particular, possibly affects gene expression through histone modification and DNA methylation changes. Under the condition of high glucose concentration and progressive oxidization associated with diabetes, it is considered that HL-CM is generated and it affects histone acetylation. Although the relationship between glycative reaction and epigenetics is a very important research area, almost nothing has been clarified. Research in the future is expected.

Epigenetics Changes Associated with Aging

It has been clarified that epigenetics plays an important role not only in diseases including cancer but also aging and senescence. For example, even though the genome sequences of identical twins are the same, it has often been observed that it's possible only one of them will develop diabetes or cancer, and their lifespans are also different because of the difference in living environments and lifestyles. These changes are considered to be caused by the epigenetic changes resulting from aging. As a result of research on the comparison of DNA methylation and histone acetylation of identical twins aged 3 years and 50 years, it was reported that although there was almost no difference between the epigenomic conditions of 3 years old identical twins, clear differences were observed between those of 50 years old identical twins [12]. It is considered that epigenetic changes accumulated differently while they were aging under different living conditions and lifestyle habits, which affected their onset of disease and life span.

Recently, a stem cell aging hypothesis has been advocated that stem cell functions deteriorate with aging, so that the structuring and maintenance of tissues become difficult. The tissue stem cells such as blood-forming stem cells and intestinal epithelial stem cell have long-term, self-replicating and multipotential functions, and they are the most important cells in structuring tissues. Because stem cells like these replicate themselves throughout life, they are considered to be good models for observing the accumulation of epigenetic changes in the process of aging. According to the research so far, the epigenetic changes in tissue stem cells over time play important roles in the individual functional deterioration caused by aging [13]. As shown in ***Fig. 4***, the epigenetic conditions of DNA methylation and histone modification in young stem cell are almost evenly maintained. However, while self-replication is being repeated with aging and the exposure to environmental factors, chronic inflammation and infection with bacteria are added to them, epigenetic changes are accumulated. Epigenetic changes are considered

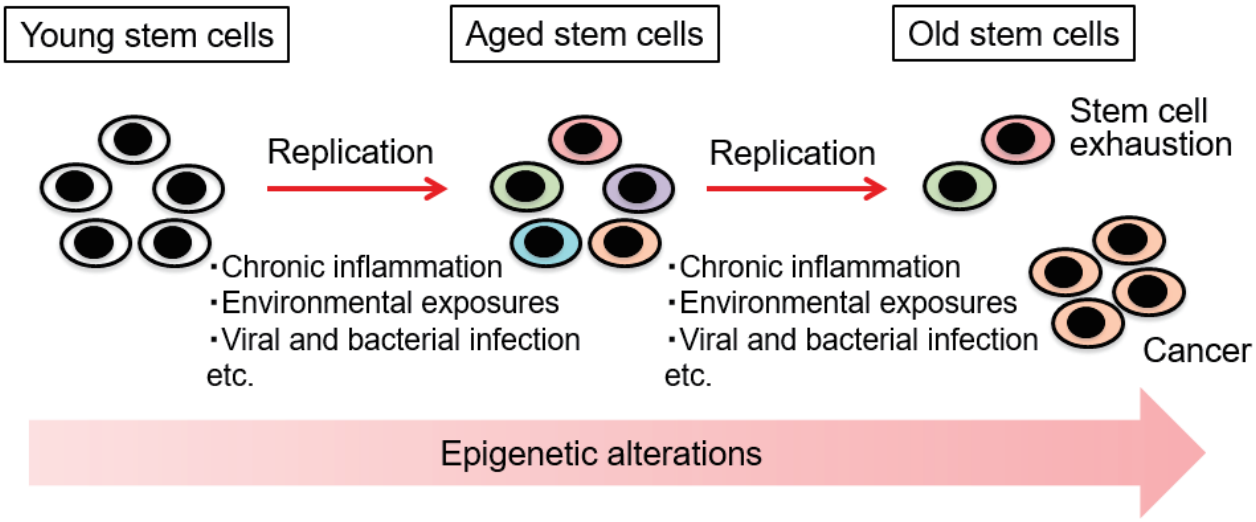

Fig. 4. **Epigenetic changes associated with aging of stem cells.**

The epigenetic conditions of DNA methylation and histone modifications are almost evenly maintained in young stem cells. While stem cells repeat self-replication with aging and the exposure to environmental factors, chronic inflammation and infection with bacteria and virus are added to it, and epigenetic changes accumulate. Epigenetic changes accelerate with aging, finally leading to tissue dysfunction associated with the depletion of stem cells and the abnormal proliferation of cancer and other diseases.

to further accelerate with aging, finally leading to the tissue dysfunction associated with the depletion of stem cells, *e.g.* reduced function of tissues and abnormal proliferation of cancer and other diseases.

Issa *et al*. discussed the relationship between aging and methylation state of the estrogen-receptor (ER) gene in large intestine mucosal tissues in a study of approximately 400 cases, and they reported that the level of methylation rose due to aging [14]. Generally, it is said that the amount of methylated cytosine contained in the total genome tends to decrease with aging. Meanwhile, it is reported that the methylation status of specific genes accelerates with aging. These changes are considered to induce chromosome instability and inactivation of tumor suppressor genes and play an important role in the onset of cancer. Toyota *et al*. precisely analyzed the methylation status of many genes and clarified that there is methylation caused by aging (type A) and cancer specific methylation (type C) in abnormal methylations [15]. As for type A genes, MYOD and N33 have been reported in addition to ER genes. There still remain unexplained points about the mechanism through which the methylation status of specific genes accelerates and further study is required.

Clarification of the Molecular Mechanism of Aging and Development of New Anti-Aging Intervention Focusing on Epigenetic Changes

Japan is a super-aging society with the elderly, 65 years or older, accounting for over 21% of the total population and the aging tendency of our country is progressing in a speed unparalleled in the world. The increased medical expenditure and care burden for the elderly are becoming a very serious problem and it is a very important task to extent healthy life expectancy through Anti-Aging intervention.

One of the factors inhibiting the progress of research in aging so far was that no good model showing the true condition of aging *in vitro* existed. In particular, we are focusing on the fact that epigenetic changes and altering gene expression accumulate in stem cells, and making efforts in clarifying the molecular mechanism of aging, using intestinal epithelial organoids established by organoid culture. Organoid culture technique is a 3-dimentional culture method of tissue stem cells and it can reproduce *in vivo* characteristics *in vitro*, so that it attracts worldwide attention. We have had success in permanently culturing and maintaining the cancer stem cells of mouse intestinal epithelial tissue and intestinal cancer tissue of $Apc^{Min/+}$ mice by the means of organoid culture [16, 17]. They also established organoids from biliary tract cancer tissue, a typical refractory cancer, and had success in culturing and maintaining the cancer stem cells derived from biliary tract cancer for more than a year [18]. Owing to the organoid culture method, it has become possible to conduct *in vitro* research with high similarity to the research with *in vivo* cells. In particular, intestinal epithelium is a dynamic tissue because intestinal epithelial stem cells existing in crypt base actively repeat self-duplication and differentiation, and all cells are replaced by new cells within 3-4 days. There are many bowel diseases such as intestinal cancer which have increased incident rates related to aging, so the organoids derived from intestinal epithelium are considered to be good models for the observation of stem cell aging.

Life phenomenon accompanied by aging is presumed to progress with multiple genetic changes related to each other in a complicated manner. If these genes are to be controlled by epigenetic changes, it is possible to control the expression of important genes relating to aging by artificially controlling epigenetic change using new low-molecular weight compounds. However, in order to apply these newly discovered epigenome drugs to clinical use for Anti-Aging, the identification of genes associated with aging and the development of gene-specific epigenetic control are required. Because the conventional DNA methylation inhibitor and histone deacetylase inhibitor fundamentally demethylate or acetylate all regions of the genome, great difficulties remain with respect to the side effects in particular. Recently, CRISPR-Cas9 technology was improved, and as a result, a groundbreaking epigenome editing technique was developed to control gene-specific epigenetic changes by recruiting the methyltransferase and demethylase regions specifically [19]. It is expected that a platform for an original Anti-Aging approach will be developed by controlling aging-specific epigenetic change, owing to epigenome editing technology. It is possible to develop the most appropriate Anti-Aging approach suitable for each case not only by epigenome editing but also by verifying the effects of the epigenetic drugs such as Nicotinamide mononucleotide (NMN) [20] of aging control factor, which is being used in clinical trials, DNA methylation inhibitor and histone methylation inhibitor.

If the development of these new Anti-Aging interventions succeeds, it is expected that it will realize the extension of healthy life expectancy and QOL and lead to the prevention of age-associated diseases such as cancer and the improvement of organ transplantation success rates in the elderly. Ultimately, it leads to the reduction of medical expenditure and care burden for the elderly and it is possible to greatly contribute to the improvement of health economics which has become an urgent problem in Japan. It is expected that the developments of epigenetics and Anti-Aging medicine lead to the realization of “rejuvenation” that people of all ages and countries have been pursuing.

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Conflict of interest

The authors claim no conflict of interest in this study.

Reference

1) Saito Y, Liang G, Egger G, et al. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human *cancer cells*. Cancer Cell. 2006; 9: 435-443.
2) Saito Y, Saito H, Liang G, et al. Epigenetic alterations and microRNA misexpression in cancer and autoimmune diseases: A critical review. *Clin Rev Allergy Immunol*. 2014; 47: 128-135.
3) Saito Y, Hibino S, Saito H. Alterations of epigenetics and microRNA in hepatocellular carcinoma. *Hepatol Res*. 2014; 44: 31-42.
4) Wu X, Zhang Y. TET-mediated active DNA demethylation: Mechanism, function and beyond. *Nat Rev Genet*. 2017; 18: 517-534.
5) Gal-Yam EN, Saito Y, Egger G, et al. Cancer epigenetics: Modifications, screening, and therapy. *Annu Rev Med*. 2008; 59: 267-280.
6) Campbell RM, Tummino PJ. Cancer epigenetics drug discovery and development: The challenge of hitting the mark. *J Clin Invest*. 2014; 124: 64-69.
7) Duthie SJ. Folic acid deficiency and cancer: Mechanisms of DNA instability. *Br Med Bull*. 1999; 55: 578 -592.
8) Kim YI. Will mandatory folic acid fortification prevent or promote cancer? *Am J Clin Nutr*. 2004; 80: 1123-1128.
9) Gibson TM, Weinstein SJ, Pfeiffer RM, et al. Pre- and postfortification intake of folate and risk of colorectal cancer in a large prospective cohort study in the United States. *Am J Clin Nutr*. 2011; 94: 1053-1062.
10) Kim YI. Folate: A magic bullet or a double edged sword for colorectal cancer prevention? *Gut*. 2006; 55: 1387-1389.
11) Ulrich CM, Potter JD. Folate supplementation: Too much of a good thing? *Cancer Epidemiol Biomarkers Prev*. 2006; 15: 189-193.
12) Fraga MF, Ballestar E, Paz MF, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci U S A*. 2005; 102: 10604-10609.
13) Issa JP. Aging and epigenetic drift: A vicious cycle. *J Clin Invest*. 2014; 124: 24-29.
14) Issa JP. CpG-island methylation in aging and cancer. *Curr Top Microbiol Immunol*. 2000; 249: 101-118.
15) Toyota M, Issa JP. CpG island methylator phenotypes in aging and cancer. *Semin Cancer Biol*. 1999; 9: 349-357.
16) Saito Y, Nakaoka T, Sakai K, et al. Inhibition of DNA methylation suppresses intestinal tumor organoids by inducing an anti-viral response. *Sci Rep*. 2016; 6: 25311.
17) Nakaoka T, Saito Y, Shimamoto Y, et al. Cluster microRNAs miR-194 and miR-215 suppress the tumorigenicity of intestinal tumor organoids. *Cancer Sci*. 2017; 108: 678-684.
18) Saito Y, Nakaoka T, Muramatsu T, et al. Induction of differentiation of intrahepatic cholangiocarcinoma cells to functional hepatocytes using an organoid culture system. *Sci Rep*. 2018; 8: 2821.
19) Morita S, Noguchi H, Horii T, et al. Targeted DNA demethylation *in vivo* using dCas9-peptide repeat and scFv-TET1 catalytic domain fusions. *Nat Biotechnol*. 2016; 34: 1060-1065.
20) Mills KF, Yoshida S, Stein LR, et al. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. *Cell Metab*. 2016; 24: 795-806.