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REVIEW

Epigenetics and cancer: towards an evaluation of the impact of environmental and dietary factors

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While the field of cancer genetics has enjoyed a great deal of attention among cancer researchers in the last few decades, the appreciation of cancer epigenetics is more recent, -owing to the fact that epigenetic mechanisms have emerged as key mechanisms in cancer development. All critical changes in cancer cells, such as silencing of tumour-suppressor genes, activation of oncogenes and defects in DNA repair, are caused not only by genetic but also by epigenetic mechanisms. Epigenetic events can affect many steps in tumour development; therefore, better understanding of epigenetic mechanisms is fundamental to our ability to successfully prevent, diagnose and treat cancer. Various environmental and dietary agents and lifestyles are suspected to be implicated in the development of a wide range of human cancers by eliciting epigenetic changes, though the contribution of epigenetic mechanisms to a given human cancer type and the precise targets of epigenetic alterations during cancer development are largely unknown. The major obstacle in establishing a relationship between epigenetic changes and exposure to dietary, lifestyle and environmental factors and cancer is the fact that studies are typically too small and lack statistical power to identify the interactions between epigenetic changes and exposures. Tremendous advances in our understanding of basic epigenetic mechanisms and rapid progress that is being made in developing new powerful technologies, such as those for sensitive and quantitative detection of epigenetic changes as well as for genome-wide analysis (epigenomics), hold great promise that these issues may be addressed in near future. Therefore, experimental evidence on the precise role of epigenetic changes induced by environment, diet and lifestyle is eagerly awaited.

Introduction

The term 'epigenetic' refers to all heritable changes in gene expression and chromatin organization that are independent of the DNA sequence itself. Epigenetic inheritance is an essential mechanism that allows the stable propagation of gene activity states from one generation of cells to the next (1). With minor exceptions (T- and B-cells of the immune system), all differentiation processes are triggered and maintained through epigenetic mechanisms.

There are three distinct classes of epigenetic information that can be inherited via chromosomes. The first class is DNA methylation, in which the DNA molecule is modified by a number of DNA methyltransferases (DNMTs). DNA methylation occurs at the 5-carbon (C^5) position of cytosine bases that are located 5′ to a guanosine base in a CpG dinucleotide (2). The methylation of DNA has multiple roles in cellular processes, including regulation of gene expression (3). The second class of epigenetic inheritance involves RNAs, which in the form of either noncoding RNA (Xist) or RNA interference (RNAi) can maintain the gene transcription state in a heritable manner (4). The third class of epigenetic information comprises histone (chromatin) modifications that encompass post-translational marking of histones. These include acetylation and methylation of conserved lysine residues on the amino-terminal portions (tails) of histones (5). A number of fascinating discoveries have led to a concept known as the 'histone code' (5–7). This hypothesis postulates that different histone modifications generate a code that is read by cellular machineries. This code thus may dictate functional outcomes by modulating different DNA-based processes, such as gene transcription and DNA repair (5,6,8,9).

One of the most remarkable recent advances is the convergence of mechanistic studies linking DNA methylation with histone modifications. These studies show that DNA methylation and histone modifications may work together to establish and maintain a repressive chromatin state and silence gene transcription (6,10–16) and that aberrant epigenetic patterns are associated with the development of human diseases, most notably cancer (13,17–20). Many excellent reviews on epigenetics have been published (1–5,21–27), and this review focuses on the recent advances in the mechanistic understanding of the contribution of epigenetic mechanisms to human cancer, with the emphasis on the role of environmental and dietary/lifestyle factors, as well as challenges and opportunities to target epigenetic changes in cancer prevention.

The fundamental role of epigenetic events in human malignancies

The growing interest in cancer epigenetics stems from the fact that epigenetic changes are implicated in virtually every step of tumour development and progression (3,22,28). Epigenetic events also play an important role in several developmental syndromes (4,22,29), cardiovascular diseases, type-2 diabetes, and obesity (30,31).

The interest in the epigenetics of cancer is further augmented by the recent realization that epigenetic changes can be exploited as a powerful tool in the clinic and as a novel approach to early diagnosis, prediction of clinical outcome and risk assessment (4,21,24,32,33). This is supported by the studies demonstrating that epigenetic changes including DNA hypermethylation are an early event in tumour development and may precede the neoplastic process (21,24,34). A distinguishing feature of epigenetic changes in comparison with genetic

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changes is that they are reversible; therefore, aberrant DNA methylation, histone acetylation and methylation are attractive targets for the epigenetic therapy. The intrinsic reversibility of epigenetic alterations also represents an exciting opportunity for the development of novel strategies for cancer prevention.

Role of aberrant DNA methylation in cancer

DNA methylation, the covalent addition of a methyl group to the cytosine base in DNA, is an epigenetic event that affects cell function by altering gene expression. In somatic cells, methylated cytosine accounts for ~1% of total nucleotides; thus, it affects ~75% of all CpG dinucleotides in the human genome (35). Strikingly, analysis of DNA methylation patterns in eukaryotes revealed the presence of so-called CpG islands, typically 0.5- to 4-kb-long guanine-cytosine-rich regions containing high relative densities of CpG sites, positioned in the promoters of many genes. It is estimated that the human genome contains ~29 000 CpG islands and that ~60% of total human genes are associated with CpG islands (2).

Previous studies have unequivocally demonstrated that DNA hypermethylation is associated with gene silencing, and that genes with high levels of methylcytosine in their promoter region are usually transcriptionally silent. DNA methylation is essential during embryonic development, and in somatic cells, patterns of DNA methylation are generally passed faithfully to daughter cells. However, aberrant DNA methylation has been associated with a large number of human malignancies, other non-neoplastic diseases and ageing (3,22,36).

Aberrant DNA methylation is found in two distinct forms: hypermethylation and hypomethylation. DNA hypermethylation is the most studied epigenetic change to date and is found in all types of cancer. Hypermethylation typically occurs at CpG islands and is associated with gene inactivation (3,22). A number of tumour-suppressor genes and other cancer-related genes, including retinoblastoma gene, *p16* gene (*CDKN2A*), *VHL, MLH1, RASSF1A, CDH1 (E-cadherin gene), LKB1, GSTP1* and *MGMT*, have been found to be silenced by promoter hypermethylation (3,22). The list of genes altered by epigenetic mechanisms is rapidly expanding (>600 genes have been reported to be modified by epigenetic mechanisms to date) and with the Human Epigenome Project in preparation (37), a more comprehensive epigenetic landscape of the human genome will be available.

Although the loss of DNA methylation was the first epigenetic alteration identified in cancer (38), global hypomethylation has been overlooked in favour of gene promoter-associated hypermethylation. However, recent studies have shown that global hypomethylation is found in virtually all human cancers (22,39–41). Although the precise mechanism by which the global loss of DNA methylation contributes to the neoplastic process is unknown, it is believed that it may act through induction of chromosomal instability and activation of cellular proto-oncogenes (22,40,42).

Role of histone modifications in cancer

In addition to aberrant DNA methylation, recent discoveries have revealed that deregulation of histone modifications and chromatin remodelling is also implicated in cancer. The histone modifications usually occur at the N-terminal 'tails' of histones protruding from nucleosomes (building blocks of chromatin). These post-translational modifications include acetylation, methylation, phosphorylation and ubiquitination. Different modifications at several amino acids at different histone tails are possible, and there is interdependence between them. For example, acetylation, methylation and ubiquitination occur at lysines, whereas phosphorylation can occur on serine or threonine residues. The importance of histone modifications is demonstrated by the fact that mechanisms involving these modifications are essential during development and that their deregulation can lead to human malignancies (17,19,20, 25,28,43). Histone proteins have thus emerged as key carriers of epigenetic information, constituting a fundamental and critical regulatory system that extends beyond the genetic information.

Interest in histone modifications has further grown over the last few years with the discovery and characterization of a large number of histone-modifying molecules and protein complexes. Different chromatin-modifying complexes act in physiological contexts to modulate DNA accessibility to the transcriptional and DNA repair machineries (9,44–47). Alterations in these chromatin-based processes could lead to mutations in oncogenes, tumour-suppressor genes or DNA repair genes resulting in genomic instability, oncogenic transformation and the development of cancer. The histone-modifying complexes include histone acetyltransferases (HATs), enzymes responsible for acetylation of the tails of core histones (47,48) and histone methyltransferases, a group of enzymes that add methyl epitope on several histone residues and are responsible for diverse functions including gene silencing and generation of heterochromatin (5).

Importantly, aberrant activity of histone-modifying factors may promote cancer development by misregulating chromatin structure and activity, an example of which is frequently found in human leukaemia (17–20). Aberrant epigenetic regulation of key cellular processes, most notably gene transcription and DNA repair, is likely to be involved in oncogenesis. However, despite the fact that progress in determining different forms of epigenetic information in chromatin has been remarkably rapid, the way histone modifications are disrupted in cancer remains largely unknown.

Epigenetic changes induced by diet, lifestyle and environmental factors in human malignancies

Epidemiological and experimental studies provide compelling evidence that either confirms or implicates various dietary and environmental factors in the development of a wide variety of neoplasms (49). Environmental factors known to play important roles in the etiology of human cancer include chemical carcinogens, such as those found in cigarette smoke, dietary contaminants, such as the aflatoxin B1 (AFB1), and physical carcinogens, such as ionizing and UV radiation (Table I). Lifestyles such as smoking, alcohol consumption, excess exposure to sunlight, fat consumption and stress may also contribute to cancer development (49–51).

Epigenetic events play a critical role in normal physiological responses to environmental stimuli that establish an appropriate gene expression pattern by altering the epigenetic state of the genome (1,52). Examples of such responses have been particularly well demonstrated in plants (53,54). Environmental and dietary factors in animals and humans inevitably affect epigenetic patterns, although a clear-cut causal relationship has yet to be established. The major obstacle in establishing such

Table I. Environmental, dietary and lifestyle factors and epigenetic mechanisms

Agent/exposure	Putative epigenetic mechanism	Gene target identified	Reference
Environment			
Tobacco smoke	Preferential binding of PAHs from tobacco to methylated CpGs	*CDKN2A, MGMT*	21,77,79,92
Nickel	Inhibition of histone acetylation, damage of core histones	*CDKN2A (p15 gene), CDKN2B, MLH1*	16,55,95–103
AFB1	Preferential binding to methylated lysines or modified histones leading to DNA damage	*RASSF1A, MGMT, CDKN2A, SNSG*	122–124
Cadmium	Inhibition of DNMTs (acute exposure), increased DNMT activity (chronic exposures)	DNMTs	61
Arsenic	Depletion of SAM leading to global hypomethylation and activation of oncogenes	*c-myc*	55,104,105
Ionizing radiation	Epigenetic (DNA methylation-mediated) silencing of key cellular regulators	p16	64
UV radiation	Global hypomethylation	—	65,66
Bacteria (*H. pylori*)	*De novo* methylation associated with chronic inflammation and cell proliferation	—	106,116,117
HPV, EBV, HBV	Methylation of viral genome, binding of viral proteins to host gene promoters, hypermethylation of host genes, changes in chromatin modification patterns, recruitment of HDACs	—	71,72,107–115
Nutrition			
Alcohol	Alcohol metabolite (acetaldehyde) acts as a cocarcinogen with HBV/HCV viruses, aflatoxin and obesity. Depletion of SAM.	*APC-1A, CDKN2D (p14 gene), CDKN2A, hMLH1, MGMT, RASSF1A, SFRP*	141–144
Dihydrocoumarin	Disruption of heterochromatic silencing, inhibition of SIRT1	—	138,139
Folate (vitamin B9) deficiency	Altered DNA methylation, altered histone modifications	—	126
Low methionine (an essential amino acid) intake	Altered levels of DNA methylation, increased binding of methyl-CpG-binding protein, altered histone modifications	—	126,128,142
Ageing and epigenetic drift	LOI, re-activation of silent alleles, re-activation of the inactive X-chromosome	*IGF2,* transposable elements	36,162–173
Transgenerational epigenetic inheritance	Histone-based germ line inheritance	—	157–159

relationship is the fact that environmental and dietary factors induce changes that are most likely subtle and cumulative, and culminate into a quantitative manifestation over a long period of time. Furthermore, the conventional epidemiology proved to be incapable of identifying dietary regimes, food components and environmental factors that induce or promote neoplastic process. This is not surprising, especially when considering the biological and chemical complexities of diet and difficulties in establishing which aspects of environment and lifestyle are hazardous. Finally, very few epidemiological and laboratory-based studies have investigated the role of epigenetic changes induced by dietary/lifestyle and environmental factors in cancer. Because the prevalence of each epigenetic mechanism is likely to vary greatly among different types of cancer, depending on the tumour-suppressor genes affected and specific dietary/environmental exposures, specific dietary/environmental factors that promote epigenetic changes in normal or cancer cells have been difficult to identify.

Nevertheless, evidence is mounting that epigenetic changes induced by environment, lifestyle and diet may play a major role in human cancer. Recent studies showed that environmental physical and chemical carcinogens, infectious agents, dietary factors and lifestyle may induce epigenetic changes leading to oncogenic transformation and cancer development (22,55,56). There is a growing list of agents in the environment and diet that have been shown to induce epigenetic changes in different model systems. For example, tobacco smoke [(57–59) and our unpublished results], arsenic (55,60), cadmium (61), nickel (55,62,63), ionizing (64) and UV radiation (65,66) are considered 'epigenetic carcinogens' (epimutagens) (Table 1). Alcohol consumption and dietary regimes may also contribute to cancer development through epigenetic mechanisms (67–70). Finally, it has been shown that certain infectious agents such as human papillomavirus (HPV) may induce silencing of host genes via an epigenetic strategy (71,72). Together, environment, diet and lifestyle contribute to cancer development by inducing both epigenetic and genetic changes that, in combination with genetic make-up, result in the disruption of key cellular processes leading to neoplastic process (Figure 1). This review summarizes our current knowledge of how the diet, environment and ageing may affect the maintenance and transmission of epigenetic patterns leading to aberrant gene transcription, integrity of genetic information and tumorigenesis.

Epigenetic mechanisms by which environment and diet contribute to tumorigenesis

In general, the degree to which environmental and nutritional factors influence tumorigenic process depends on the presence of specific hazardous food components, food composition, dietary regime and amount and duration of exposure. However, the degree to which hazardous exposures affect carcinogenesis largely reflects variation in susceptibility to a given dietary or environmental exposure. Individual susceptibility is likely to

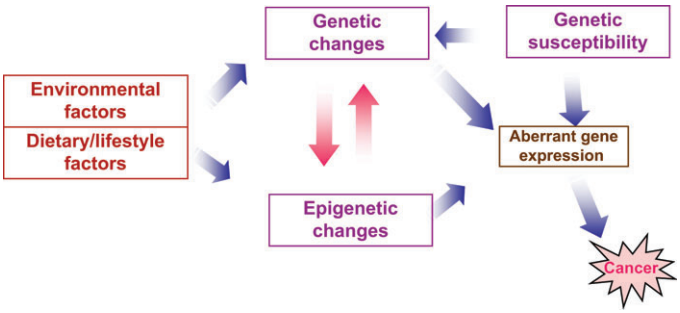

Fig. 1. Impact of epigenetic and genetic alterations induced by environmental, dietary and lifestyle factors in cancer. Recent discoveries indicate that epigenetic and genetic alterations either individually or in combination can be accounted for most human cancers. Epigenetic and genetic changes induced by adverse stimuli in the environment and diet in conjunction with genetic make-up affect critical cellular processes such as gene expression, leading to oncogenic transformation and tumour development.

depend on the epigenetic make-up dictating an individual's response and adaptation mechanisms, including DNA repair, carcinogen detoxification, metabolic response and apoptosis. Differences in individual susceptibility may be attributed to patterns of both histone modifications and DNA methylation in addition to genetic make-up.

Generally, environmental and dietary/lifestyle factors that are capable of inducing tumour development by eliciting epigenetic changes can be broadly divided into two groups: (1) agents that induce changes, either directly or indirectly, in genomic DNA and (2) agents that affect critical cellular regulatory processes, such as gene transcription, DNA damage detection and signalling, DNA repair, cell cycle control and cell death. The agents in the first group may alter the level or pattern of DNA methylation triggering changes in DNA (e.g. mutational events). This was exemplified by a study showing that the mismatch repair gene *MHL1* is frequently hypermethylated in sporadic tumours exhibiting microsatellite instability (73,74). Similarly, silencing of *MGMT*, the DNA repair gene encoding protein responsible for the removal of carcinogen-induced O^6-methylguanine adducts from DNA (which if left unrepaired results in G to A transition mutation), appears to increase the mutation rate in critical cellular regulators, including tumour suppressors and oncogenes (75,76). Therefore, environmental and dietary exposures that alter either the expression or the activity of enzymes involved in *de novo* DNA methylation (DNMT3A and DNMT3B) and/or the maintenance of DNA methylation (DNMT1) may predispose to mutational events. Alternatively, different agents in the environment and diet may induce mutational events through preferential binding to hypermethylated DNA. This was shown to be true for benzo(a)pyrene diol epoxide (BPDE) (77), a carcinogen from tobacco smoke that exhibits preference for methylated CpG sites, resulting in formation of DNA adducts and G to T transfersions, often found in cancers of the aero-digestive tract in tobacco smokers (78,79).

The agents in the second group may alter the pattern of chromatin modifications (histone code) in a transient manner and are likely to induce changes in key cellular processes including gene transcription, DNA damage response and DNA repair. Primary epigenetic targets for environmental/dietary factors in this group may be the proteins and protein complexes responsible for histone modifications such as HATs and histone deacetylases (HDACs), whose activities are often found deregulated in cancer (19,20,25). Recent studies showed that HATs are involved in the process of DNA repair (9,44,46,80), suggesting that even moderate and transient inhibition of HAT activity induced by environmental and dietary exposures may compromise DNA repair, leading to mutation fixation and genomic instability. Similarly, HDAC was shown to be required for efficient DNA repair (45,81), suggesting that the removal of histone acetylation is required for restoration of normal (default) chromatin structure following the completion of DNA repair. A tight regulation of HAT and HDAC activity is thus essential for proper regulation of gene transcription and DNA repair. Reduced levels of histone acetylation or enhanced histone deacetylation may result in the compaction of chromatin, blocking access of transcription factors to DNA and/or impeded progression of RNA polymerase. Therefore, different environmental and dietary factors may transiently alter chromatin-modifying/remodelling activities and alter patterns of histone modifications impeding DNA repair and other chromatin-based processes.

Another possible epigenetic 'target' of adverse environmental and dietary exposure may be general methyl-C-binding proteins, a group of proteins (including MBD1, MBD2, MBD3, MeCP2 and KAISO) that bind to methylated CpG sites (1,2,25). Some members of this family, exemplified by MeCP2, were found to bind and recruit HDAC to chromatin (13,82). Changes in MeCP2 protein stability and function elicited by the hazardous agents in diet and environment may thus affect normal gene transcription, leading to aberrant cell proliferation and cancer. Given that histone modifications and DNA methylation appear to work together to establish a permissive or repressive chromatin state, agents in the environment and diet that affect one of these intimately linked and self-reinforcing mechanisms would inevitably affect the other. Although poorly understood, the molecular mechanisms by which epigenetic carcinogens in environment and diet may exhibit adverse effects on histone modifications are beginning to emerge (see below). Several recent studies have examined the effect of specific environmental carcinogens on histone modifications and suggest that these agents may affect the pattern of histone modifications through different mechanisms (Table I).

Epigenetic carcinogens in the environment

A number of epimutagens in the environment and diet have been found to alter epigenetic patterns, although much remain unknown. Recent studies have examined the effect of specific environmental carcinogens on both DNA methylation and histone modifications and provided what is so far a rather sketchy picture of underlying mechanisms (Table I).

Tobacco smoking

Early epidemiological studies established a strong association between tobacco smoking and lung cancer in men (83–85). Although our knowledge on the genetic changes associated with tobacco smoking is relatively extensive (86–88), until recently we knew little about the epigenetic events in tobacco-associated cancer. The mechanisms by which tobacco smoke alters epigenetic patterns and silences the gene expression are largely unknown. Tobacco smoke is a complex aerosol and the International Agency for Research on Cancer has identified no <44 substances in tobacco smoke for which there is sufficient evidence for carcinogenicity in either humans or laboratory

animals (89), although recent studies have indicated that among 4800 identified compounds in tobacco smoke, as many as 69 may be carcinogens (89,90). The great deal of experimental studies on lung carcinogenesis by tobacco smoking has been focused on polycyclic aromatic hydrocarbons (PAHs), mostly benzo[a]pyrene, considered the most carcinogenic (91). Although the mechanism is unclear at present, epigenetic targets of the PAHs from tobacco smoke may induce DNA damage through preferential binding to methylated CpG sites, a phenomenon already demonstrated for BPDE, a carcinogen found in tobacco smoke (77,79).

In addition, several recent studies described the hypermethylation and silencing of several genes in lung cancer associated with smoking. The genes frequently altered by promoter hypermethylation in lung cancers of smokers are p16 and MGMT (21). Interestingly, in addition to the p53 pathway, the p16 gene appears also to be a major target of genetic alterations in the pathogenesis of lung cancers in smokers (92). Therefore, tobacco smoke exerts its carcinogenic effect by inactivating key tumour-suppressor pathways through both genetic and epigenetic mechanisms.

Different components in tobacco smoke may also induce histone changes and alter histone code; however, this possibility remains to be tested. Regardless of the underlying mechanism, recent studies have shown that aberrant promoter methylation of several genes including p16 and MGMT can be detected in DNA from sputum in not $<100\%$ of patients with lung squamous cell carcinoma even up to 3 years before clinical manifestation of cancer (21,24,93). Therefore, it appears that the promoter hypermethylation is an early event in the development of lung cancer and may constitute an excellent biomarker for early diagnosis and risk assessment.

Nickel

Studies with nickel, a potent human and animal carcinogen (94), have been very useful in illustrating how environmental and occupational exposures may severely perturb epigenetic information, notably that in chromatin (55). Broday *et al*. (95) found that nickel induces silencing of gene transcription by changing patterns of histone acetylation in a yeast model (*Saccharomyces cerevisiae*). Detailed analyses revealed that nickel exposure decreased acetylation levels at specific lysine residues including lysine 12 and 16 of histone H4 (96). Interestingly, other non-lysine residues of histone H4, namely, histidine at position 18, appear to be a preferential binding site for nickel (97). It is proposed that nickel molecules bound to histidine 18 affects accessibility of neighbouring lysines to HAT complexes. Biochemical studies showed that cultured cells exposed to nickel exhibited cleavage of the C-terminal tail of the variant of histone H2A, as well as truncation, deamidation and oxidation of histone H2B (98–100). Recent studies identified additional chromatin changes in cells exposed to soluble nickel compounds including the loss of acetylation of H2A, H2B, H3 and H4, increased H3-K9 dimethylation and increased ubiquitination of H2A and H2B (101).

Studies using the HDAC inhibitor trichostatin A (TSA) showed HDAC inhibition during and after nickel exposure counteracted nickel-induced gene silencing and cellular transformation (102,103). Similar observations were obtained for several cancer-related genes including p16, p15 and MLH1 (16). These results illustrate the fact that environment and diet may induce a number of distinct reversible changes at the level of histone proteins, representing an interesting target for therapeutic intervention and the development of preventive strategies.

Arsenic

Environmental agent arsenic, a potent human carcinogen found as a contaminant in food and water, may also exhibit its carcinogenic activity by inducing epigenetic changes (55). Chronic exposure to arsenic compounds has been found to promote oncogenic transformation of cultured cells (104). Transformed phenotype was associated with changes in expression of several genes including the proto-oncogene c-myc (105). Although the precise underlying mechanism is unclear, it has been proposed that exposure to arsenic results in the depletion of cellular S-adenosylmethionine (SAM), a metabolite needed for both the metabolism of arsenic and the process of DNA methylation (104). Therefore, long-term exposure to arsenic may result in the depletion of cellular pools of SAM leading to the global DNA hypomethylation, a phenomenon believed to promote the activation of oncogenes (22).

Viruses and bacteria

Environmental agents including viruses, such as HPV, Epstein–Barr virus (EBV) and human hepatitis virus (HBV), and bacteria such as *Helicobacter pylori* may also alter expression of host genes via an epigenetic strategy (71,72,106,107). Epigenetic mechanisms including DNA methylation and chromatin modifications are known to regulate viral gene expression (108). It has been shown that methylation of integrated HPV genome in primary cancers and cervical cancer cell lines inhibits the transcription of the most viral genes (107). Interestingly, CpG methylation appears to correlate with HPV pathogenesis, suggesting that methylation of HPV DNA is implicated in the development and progression of cervical cancer (107,109,110). EBV genomes are also subject to host cell-dependent epigenetic modifications including DNA methylation, binding of regulatory proteins and histone modifications, and different EBV latency types are associated with distinct viral epigenotypes (72). Associations between EBV and HBV infection and promoter hypermethylation of several genes were commonly found in several cancers including hepatocellular, gastric and nasopharyngeal carcinoma (111–113).

The mechanism by which viruses induce aberrant epigenetic states in host genome sequences is poorly understood. The first possibility is that DNA methylation onto the neighbouring host genome spreads from integrated viral genome. However, DNA methylation in certain virus-associated cancers (e.g. cervical cancers associated with HPV infection) appears not to be specific for viral DNA, but rather occurs as a frequent event throughout the host genome (114,115). Therefore, it is more likely that viral proteins bind to promoter regions of host genes, preventing transcription factors from accessing their binding sites. Alternatively, viral proteins may interact with methyl-CpG-binding proteins that recruit HDAC in association with chromatin. Consequently, alterations in histone modifications lead to changes in the chromatin configuration that are refractory to transcription.

In addition to viruses, a recent study established the correlation between *H. pylori* infection and high levels of aberrant DNA methylation in gastric cancer (106). *Helicobacter pylori* infection may induce various degrees of methylation of CpG islands in promoters of several genes in gastric mucosa, which appears to reflect gastric cancer risk. The mechanism underlying *H. pylori*-induced changes in DNA methylation is

unknown. While in animal models *H. pylori* by itself rarely induces gastric cancer, its presence seems to enhance cancer incidence after initiation with mutagens such as *N*-methyl-*N*-nitrosourea (116), suggesting that DNA methylation changes induced by *H. pylori* have tumour-promoting effect. Given that *H. pylori* infection in turn induces chronic inflammation and cell proliferation (117), it is possible that these events induced by bacterial infection trigger *de novo* hypermethylation (117–119). In addition, the suppression of gene expression in the host genome, a phenomenon observed during inflammation, may promote DNA hypermethylation (120,121).

Aflatoxin

Several studies have addressed whether AFB1, a naturally occurring environmental carcinogen produced by certain species of the fungus *Aspergillus* and found on corn and other crops, can exhibit its carcinogenic effects through epigenetic mechanism. These studies established a strong relationship between AFB1 exposure and methylation status in cancer-related genes including RASSF1A (122), MGMT (123) and p16 (124) in tumour tissues and plasma DNA of hepatocellular carcinoma (HCC) patients. Using a human hepatoma-derived cell line (HepG2) as an *in vitro* model, it was demonstrated that AFB1 strongly induces the expression of the SNCG gene, a known target of epigenetic changes in early stages of HCC (124). Although the precise mechanism by which AFB1 alters epigenetic states is unclear, AFB1 may bind preferentially to methylated CpG sites and/or specific structure in chromatin (for example, specific histone lysines modified by acetylation or methylation), inducing the damage of DNA and histones. Analogous mechanisms have been observed for DNA methylation-associated potentiation of carcinogen-DNA adduct formation at specific codons in the K-ras gene (125) and specific histone damage induced by nickel binding (55), respectively.

Diet and epigenetic changes in cancer

Although relatively little is known regarding the direct effects of dietary factors on epigenetic changes, there are examples where this relation is better understood (Table 1). The most studied and among the best understood is the relationship between dietary methyonine and DNA methylation (126). As an essential amino acid, methionine plays the central role in the epigenetic regulation by serving as methyl donor for methylation reactions. In the process of cytosine methylation, DNMT enzyme converts SAM to S-adenosylhomocysteine (SAH); therefore, an optimal supply of SAM or removal of SAH is essential for a normal establishment of genome-wide DNA methylation patterns. CpG methylation patterns are largely erased in the early embryos and then re-established in a tissue-specific manner (127). Therefore, early embryonic development may represent a sensitive stage, and dietary and environmental factors that affect DNA methylation reaction and the activity of DNMTs may result in permanent fixation of aberrant methylation patterns.

This idea is supported by a study demonstrating that dietary methyl supplementation during early embryonic development in mice increases methylation levels at specific genes resulting in permanent changes in gene expression (128). This gene-specific hypermethylation was explained by the nutrition, which may influence specific loci susceptible to the establishment of epigenotype with a dynamic stochasticity. However, even in adulthood, the homeostasis of most tissues is dependent on constant self-renewal involving differentiation of tissue-specific stem and progenitor cells. This process requires the establishment of tissue-specific patterns of DNA methylation and histone modifications. Furthermore, in postnatal development and adulthood, established patterns of DNA methylation and histone modifications must be maintained through multiple mitotic divisions; therefore, inappropriate quantities of methionine, other food components and environmental agents may affect normal patterns of DNA methylation and histone modifications. In this respect, it is interesting to note that in adult men with hyperhomocysteinemia, a disorder occurring in several genetically determined and -acquired diseases with uraemia, treatment with high doses of folate increases methylation levels at specific genes and restores normal expression (129). In addition to methylation of DNA, methylation of histones, a distinct epigenetic mechanism dependent on 1-carbon groups, may be affected by consuming excessive levels of specific nutritional factors (126). Therefore, nutrition and environment are also likely to directly or indirectly (through changes in DNA methylation) affect histone modifications such as histone methylation. However, this hypothesis has not yet been tested.

Another potential mechanism by which environmental and dietary exposures affect the epigenome may involve transposable elements. Transposons are groups of mobile genetic elements that, when activated, may cause genetic mutations and transcriptional noise (130,131). Transposable element-related sequences are numerous in the mouse and human genomes [e.g. the Alu family alone consists of several hundred thousand elements (132)], and are shown to be heavily methylated and transcriptionally silent in somatic cells. Although it is well documented that the activation of transposable element-derived promoters may be a consequence of perturbed DNA methylation, transposable elements were shown to be activated by different kinds of cellular stress (133,134). Therefore, stress induced by environmental and dietary agents may activate transposable elements, leading to altered establishment and maintenance of epigenetic states.

Different classes of HDACs may also be altered by environmental and dietary agents. Interestingly, three dietary chemopreventive agents, butyrate, diallyl disulfide and sulforaphane, also have HDAC inhibitory activity. This is highlighted by a recent study demonstrating that resveratrol, a molecule produced by a variety of plants and the most potent inhibitor of SIRT1, a member of the sirtuin family of NAD-dependent deacetylases (135), improves health and extends the lifespan of mice on a high-calorie diet (136). Since high caloric intake and obesity are often associated with other age-related diseases including cancer, it is possible that the environment and diet may influence neoplastic process by altering the activities of sirtuins. Consistent with this idea, inhibition of SIRT1 enzyme by different means affected key phenotypic aspects of cancer cells (137). Furthermore, dihydrocoumarin, a compound found in *Melilotus officinalis* (sweet clover) that is commonly added to food, was shown to disrupt heterochromatic silencing and to inhibit SIRT1 deacetylase activity (138). Although sirtuins are believed to exhibit their activity mainly through deacetylation of non-histone proteins (139), recent evidence suggests that they are important for the maintenance of histone acetylation patterns (such as histone H4-K16 and H3-K9 acetylation) at endogenous promoters (137). This observation argues that sirtuins

may also affect normal gene expression through epigenetic regulation of chromatin states, linking epigenetic changes associated with ageing and those found in tumours. These studies highlight the fact that human diet contains a number of different substances that when present at higher concentrations and over longer time period are capable of altering histone acetylation and chromatin states, contributing to the phenotypes associated with ageing and cancer. Therefore, the effects of environmental and dietary factors on histone modification patterns and underlying mechanisms will be an important area in nutritional and environmental epigenetics and epidemiology.

Alcohol

Alcohol is associated with several human cancers, notably liver cancer and head and neck cancer. While the most likely contributing effect of alcohol to the development of liver cancer is the induction of a well-known precancerous liver lesion, cirrhosis (140), the targets of alcohol in head and neck cancer are less clear. Alcohol is known to enhance the effects of environmental carcinogens directly and by contributing to nutritional deficiency and compromising the organism's tumour defence. Alcohol may also act as a potent cocarcinogen, and exhibits strong synergistic effects with other carcinogens including HBV and hepatitis C virus (HCV), aflatoxin and obesity. The main metabolite of alcohol, acetaldehyde, causes liver injury including DNA damage. Alcohol drinking also affects nutrition and metabolism of different critical compounds, notably vitamins.

Several studies have provided evidence that alcohol consumption is associated with different epigenetic changes in human cancer. In a large epidemiological study (the Netherlands Cohort Study on diet and cancer), analysis of DNA methylation showed that the prevalence of promoter hypermethylation of several genes including *APC-1A, CDKN2D, CDKN2A, hMLH1, MGMT* and *RASSF1A* was higher in colorectal cancer patients with high alcohol (and low folate) intake than among colorectal cancer patients with high folate/low alcohol intake (141,142). In addition, the study of human head and neck squamous cell carcinoma showed that the promoter hypermethylation of *MGMT* gene and the genes known to regulate the WNT pathway occurs more frequently in both heavy and light drinkers compared to non-drinkers (143,144).

The mechanism underlying the epigenetic changes caused by alcohol abuse may also involve SAM. This small metabolite is regenerated from demethylated SAM via the methionine cycle, which involves folate. Therefore, imbalance of this cycle through alcohol consumption may result in depletion of SAM and aberrant epigenetic patterns. In addition, it was shown that the human class I alcohol dehydrogenase (ADH) genes may be regulated by epigenetic mechanism. The class I ADH genes were found to be repressed in human hepatoma cell lines, but could be reactivated after the treatment with HDAC inhibitor (TSA) or DNA demethylating agent 5-aza-2′-deoxycytidine (145). This suggests that epigenetic changes associated with alcohol-metabolizing genes may also enhance other toxic effects of alcohol on different organs, most notably the liver, including steatosis, cirrhosis and ultimately hepatic tumorigenesis.

Studies of environment/diet and epigenetic states in mouse models

In addition to the complex nature of nutrition/lifestyle and environment, until recently the major obstacle in studying the effects of these factors on epigenetic states has been the lack of appropriate animal model systems. The yellow *agouti* mouse appears to represent a sensitive indicator of diet-induced changes in epigenetic information and has proven to be very useful in studying the role of nutrition on epigenotype (128,146,147).

The coat colour gene *agouti* in mice encodes a signalling molecule that stimulates the production of yellow pigment. *Agouti* yellow alleles (such as A^{vy} and A^{IAP}) arise spontaneously following genetic and epigenetic perturbation of the gene induced by insertion of retrotransposon particle in the locus (148–150). These changes are reflected in coat colour ranging from yellow to mottled to wild-type *agouti*. Interestingly, yellow mice are susceptible to obesity and tumour development. It is known that variation in coat colour is a consequence of the changes in methylation levels of inserted transposons. The coat colour of the *agouti* (A^{vy} and A^{IAP}) mice may thus be used as an easily detectable and sensitive readout of the methylation states of adult mice. These models have been exploited to investigate the influence of maternal diet during pregnancy on the phenotype of *agouti* mice (147,151). Wolff *et al*. (147) found that feeding pregnant female mice a diet supplemented with high doses of folic acid, choline and vitamin B12 shifts the coat colour of their offspring.

In another study, Cooney *et al.* (151) showed that female mice fed with high levels of dietary methyl supplements before and during pregnancy changed the phenotype of the offspring. Subsequent studies demonstrated that this diet-induced phenotypic change is caused by hypermethylation of the *agouti* locus (128,151). These findings led to the suggestion that changes of epigenetic states induced by nutritional factors are not limited to the *agouti* locus but are likely to be a general feature of other metastable epialleles (126,152). In agreement with these observations, Gaudet *et al*. (153) found that haploinsufficiency of the DNMT1 impaired DNA methylation at the *agouti* locus resulting in stable transcriptional activation of the locus in adult mice. A recent study showed that another murine metastable epiallele, *axin fused* (*Axin(Fu)*) (154), similarly exhibits epigenetic plasticity to maternal diet (126). The authors found that in female mice diet containing high levels of methyl donors before and during pregnancy increased DNA methylation at *Axin(Fu)* and reduced the incidence of tail kinking associated with the *Axin(Fu)*/+ allele. These observations also suggest that exposures to hazardous nutritional and environmental factors during early embryonic life could alter epigenetic states and consequently induce permanent changes in gene expression and the phenotype in adulthood.

Environment, diet and transgenerational epigenetic inheritance

Epigenetic modifications are believed to be cleared on passage through the germ line in mammals, so that only genetic traits are inherited. However, there is increasing evidence in both animals and plants that epigenetic marks are not completely cleared between generations (155,156). Incomplete erasure at genes associated with a measurable phenotype results in unusual patterns of inheritance from one generation to the next, termed transgenerational epigenetic inheritance. While transgenerational influence of maternal diet is well recognized, recent studies have suggested that epigenetic states can be inherited transgenerationally after paternal transmission. For example, the presence or absence of DNA methylation

associated with the characteristic phenotype of the murine *Axin(Fu)* allele, a kinked tail, can be inherited transgenerationally after both maternal and paternal transmission (157).

Consistent with these findings, a recent epidemiological study suggested that environmental and dietary exposures in men may influence health and susceptibility to diseases in following generations. Pembrey *et al.* (158) have studied the effects of parental diet and lifestyle on the development and health of the following generations in two general populations. The first was a contemporary population (ALSPAC) in which parental history was taken before the birth of the study child and followed by prospective collection of data. The second, a three-generation population (in Överkalix, a parish in northern Sweden), was analysed using historical records. The authors observed transgenerational effects with dietary exposure of grandparents. Interestingly, it was proposed that there may be sex-specific, male-line transgenerational responses in humans (158,159). For example, a transgenerational effect of a father's mid-childhood smoking on body mass index in his child at young age was observed. Therefore, it appears that human germ line has 'captured' information through epigenetic states about the ancestral environment and passed it to the next generation.

An important support for this hypothesis comes from an elegant study on *Drosophila melanogaster* in which altered epigenetic states induced by specific stimuli could be transmitted to subsequent generations through altered chromatin states (160). This study demonstrates that patterns of gene expression regulated by epigenetic information involving chromatin factors could be inherited through the germ line. Moreover, it suggests that epigenetic mechanisms involving histone-based germ line inheritance may be subject to selection and morphological evolution (160,161). To what degree the parental environmental and dietary exposures contribute to cancer incidence through transgenerational epigenetic inheritance is unknown. Future studies are required to substantiate these observations and to define the underlying mechanism.

Epigenetic drift in ageing and cancer

Ageing, one of the most significant risk factors for human neoplasms, may also act through epigenetic mechanisms to predispose to cancer. A strong association has been established between epigenetic alterations, both global hypomethylation and local hypermethylation, and ageing in cells and animals. A progressive decrease in global methylation has been observed in cultured fibroblasts (36,162) and aged animals (163,164). Altered methylation in specific genes such as *IGF2* and transposable elements (165) has also been found to be age-related, suggesting that epigenetic abnormalities accumulate over time (166). Epigenetic changes as a function of age are particularly well studied in human colorectal mucosa. It has been suggested that DNA methylation-associated inactivation of the specific genes in ageing colorectal mucosa could be one of the earliest events that predispose to sporadic tumours (167). Genomic imprinting, a form of gene silencing that is epigenetic in origin, may also be disrupted over time. In animal models, loss of imprinting (LOI) was frequently found in aged animals, leading to re-activation of typically silent allele (168), in agreement with the study showing the age-related re-activation of the inactive X-chromosome (169). Given that LOI correlates with the susceptibility to colorectal cancer in humans (170) and mice (171), age-related LOI may be an important contributing factor to the development of sporadic cancers. Strikingly, the analysis of LOI and re-activation of the inactive X-chromosome in inbred mice revealed much higher frequencies of these epigenetic events (169) than frequencies reported for genetic changes (172). These observations may suggest that epigenetic changes may be even more important to age-related cancers than genetic mutations.

In contrast to mouse-inbred strains, in humans there is a high degree of genetic polymorphism between individuals; therefore, the effects of ageing on the maintenance of epigenetic states in men have been more difficult to establish. This obstacle has been circumvented in a recent study of identical twins (173). Monozygous twins are considered genetically identical and are thus ideal for studying the effects of environmental and dietary factors on human health and disease. Using a large cohort of identical twins, Fraga *et al.* (173) demonstrated that although identical twin pairs are epigenetically indistinguishable at early life, in later life, they exhibit remarkable differences in epigenetic patterns (173). Over time, identical twins accumulated significant differences in both global levels and patterns of methylation as well as patterns of histone acetylation. Moreover, the older twins displayed great differences in gene expression profiles. Importantly, older twin pairs who lived apart longer also differed most with respect to DNA methylation and histone acetylation levels and at specific loci also displayed the most different gene expression profiles (173). These findings argue that the epigenetic drift arising during postnatal life may be attributable largely to environmental and dietary factors.

Because the transmission and maintenance of epigenetic states is mediated by different mechanisms involving highly complex and interdependent processes such as DNA methylation and different forms of histone modifications, the actual causes of the differences in epigenetic patterns even in identical individuals are not readily discernable. While environmental and dietary stimuli such as tobacco smoking, alcohol consumption and nutritional regimes are likely to contribute to changes in epigenetic states over time, intrinsic factors may also contribute significantly to epigenetic drift. For example, internal factors such as stochastic defects in transmitting epigenetic patterns over many cell divisions may lead to accumulation of epigenetic alterations. To what degree the adverse influences of the environment and diet and the intrinsic defects in the complex mechanism of transmission and maintenance of epigenetic patterns contribute to the epigenetic drift associated with different types of human cancer remains to be addressed. Given that all elements of epigenetic drift, including the loss of global methylation, localized hypermethylation and loss of histone acetylation, arise in a gradual manner, epigenetic changes represent an excellent target for preventive and therapeutic strategies. This is exemplified by the studies demonstrating that the reduction of DNA methylation prevented the formation of intestinal adenomas in a tumour-prone mouse model (174) and reactivated the expression of genes that have undergone epigenetic silencing (3,4). Because epigenetic drift may contribute to the development of chronic diseases including cancer, strategies applying different drugs and changes in diet and lifestyle might be highly beneficial in preventing/reversing epigenetic alterations and counteracting the disease.

Concluding remarks

Both the scientific and medical communities now recognize the importance of epigenetic changes in cancer; however, the

precise contribution of epigenetic mechanisms and cellular targets of epigenetic alterations in human cancers are largely unknown. A number of recent breakthroughs in the field of epigenetics have enhanced our understanding of epigenetic changes in normal cellular processes and abnormal events leading to oncogenesis. Interest in cancer epigenetics has dramatically grown over last few years with the realization that exploiting epigenetic changes has tremendous potential in the prevention and treatment of cancer.

The study of the role of epigenetic changes induced by environmental, dietary and lifestyle factors is in its infancy, and little is known of the precise contribution of epigenetic mechanisms to different types of human cancers and direct gene targets of epigenetic alterations induced by adverse stimuli in the environment and diet. While there is accumulating evidence showing that aberrant DNA methylation may result from adverse exposures to epimutagens, there is a paucity of evidence regarding the effects of stimuli causing heritable changes in epigenetic information stored in histones, owing to the fact that this is a new and largely unexplored field of cancer epigenetics. Although it seems inevitable that perturbations in histone modifications are induced by dietary and environmental factors that contribute to the development of human cancer, a formal proof of such a relationship remains to be established. However, emerging powerful technologies for detection of epigenetic changes (27,175) and recent progress in the field of epigenomics will further advance the capacity to address key issues. Such tools are already in use to characterize tumour samples in robust and high-throughput settings.

In addition, large cohort and case–control studies offer some of the most exciting opportunities to study the contribution of epigenetic events induced by the diet and environment to human cancer. Such examples are the European Prospective Investigation into Cancer and Nutrition, a large prospective cohort study designated to investigate the relationship between diet, various lifestyles and the incidence of cancer in 10 European countries (176), and the case–control study on lung and upper-aerodigestive tract cancers in Central and Eastern Europe (177–179). These multicentre studies, whose recruitment has been recently completed, boast a large sample size of several thousand subjects and represent unique possibilities to identify which dietary and environment stimuli and lifestyle practices may exert carcinogenic effects through epigenetic changes.

Epigenetic alterations in comparison with genetic changes are reversible and are typically acquired in a gradual manner. These features offer an enormous potential for prevention strategies. Based on quantitative estimates, over two-thirds of the cancer incidence accounted for by environmental and dietary factors (50); therefore, the majority of cancers are potentially avoidable. It remains to be established which proportion of the variation in cancer incidence accounted for by epigenetic changes induced by environmental and dietary factors. Therefore, the near future is likely to bring long-awaited answers on the impact of aberrant epigenetic information caused by diet and environment and provides important information for the discovery of new biomarkers and the development of novel strategies for cancer prevention, a major public health priority in the 21st century.

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