Abstract

It is becoming increasingly apparent that toxicant-induced changes in epigenetic status, particularly DNA methylation patterns, may play a role in some mechanisms of toxicity. Here, we discuss briefly the evidence that alterations in DNA methylation accompany, and may even promote, carcinogenesis induced by non-genotoxic chemicals. We also address recent findings indicating that the availability of dietary methyl donors can modulate DNA methylation levels and precipitate adverse effects.

Keywords: Epigenetic; DNA methylation; Chromatin; Non-genotoxic carcinogen; Estrogen; Nutrition

1. Introduction

The term epigenetics describes the study of heritable alterations in gene expression that occur in the absence of changes in genome sequence. This can be contrasted with genetics, which deals with the transmission of information based on differences in DNA sequence. The best-studied mechanism of epigenetic regulation involves the methylation of DNA at cytosines, to form 5-methyl cytosine (5-mC). DNA methylation has been implicated in a variety of biological processes, but the molecular mechanisms by which it controls genome function have only recently begun to be elucidated. It is now clear that cytosine methylation plays a pivotal role in gene silencing, X chromosome inactivation, genomic imprinting, and embryonic development (Reik et al., 2001; Jaenisch and Bird, 2003; Grewal and Moazed, 2003). There has also been an explosion of interest in the role played by epigenetics in human diseases such as cancer, neurological disorders and diabetes (Petronis, 2001; Feinberg et al., 2002). This has been accompanied by the increasing realisation that epigenetic mechanisms may underpin adverse responses to certain chemicals (reviewed by Goodman and Counts, 1993). Here, we review concisely the evidence that epigenetic mechanisms, and in particular alterations in DNA methylation, mediate certain mechanisms of toxicity. For more thorough reviews on this topic, see Murphy and Jirtle (2000), Watson and Goodman (2002a).

2. DNA methylation changes in human cancers

5-mC represents 2–5% of all cytosines in mammalian genomes and is found primarily on CpG dinucleotides (Millar et al., 2003). Clusters of CpG sequences (known as CpG islands) tend to be found in the vicinity of the 5′ ends of genes and are usually
 unmethylated. However, methylation of a proportion of cytosines in these CpG islands can occur in a life-stage- and cell type-dependent manner and usually correlates with silencing of the adjacent gene. Feinberg and Vogelstein (1983) were the first to show that cancer cells sometimes have abnormal patterns of DNA methylation. This suggested, enticingly, that perturbations in DNA methylation might initiate carcinogenesis. Specifically, hypomethylation or hypermethylation of CpG islands might lead to the constitutive expression of oncogenes or the silencing of tumour suppressor genes, respectively. An abundance of experimental data support this hypothesis: more than 50 genes have been shown to be abnormally methylated in cancers, including the \textit{k-ras} oncogene implicated in the development of colon tumours (Esteller, 2002). It has also been suggested that a decrease in the overall level of DNA methylation, associated with many cancers, directly contributes to the transformed state by mobilizing usually silent transposable elements (Carnell and Goodman, 2003), which may cause chromosomal instability. However, it must be pointed out that although it is clear that tumour cells often harbour epigenetic aberrations, it is not clear whether DNA methylation changes cause cancer, or arise as a consequence of the transformed state (Baylin and Bestor, 2002).

3. Do epigenetic changes play a role in chemical carcinogenesis?

3.1. Non-genotoxic chemical carcinogens

Evidence is accumulating that environmental influences, such as xenobiotic exposure and diet, can alter DNA methylation levels in rodents, sometimes at specific gene loci. A tumour-inducing dose of the non-genotoxic hepatocarcinogen, phenobarbital (PB), reduced the overall level of liver DNA methylation in a tumour-sensitive (B6C3F1) mouse strain (Counts et al., 1996). This change was reversible: methylation levels returned to normal following a 4-week recovery period. Importantly, the same dose of PB did not alter global methylation levels in a more tumour-resistant strain (C57BL/6), although the compound increased hepatocyte proliferation in both strains (Counts et al., 1996). In a similar study, Watson and Goodman (2002b) used a PCR-based technique to measure DNA methylation changes specifically in GC-rich regions of the mouse genome. They found that, in these areas of the genome, exposure to PB caused an increase in methylation in dosed animals compared with control animals. Again, the change was more pronounced in tumour-prone C3He and B6C3F1 strains than in the less sensitive C57BL/6 strain.

In a further study, using the SENCAR mouse model of tumour initiation/promotion (Slaga et al., 1996), another tumour-promoting agent (cigarette smoke condensate, CSC) altered methylation globally and in GC-rich regions in a dose- and time-dependent fashion (Watson et al., 2003). In this study, CSC treatment promoted tumour formation following initiation with dimethylbenz[a]anthracene (DMBA), and changes in the methylation of GC-rich regions were more pronounced in tumours than in healthy tissue (Watson et al., 2003). The initial CSC-induced methylation changes were reversed following a recovery period. Other studies have revealed that chemical carcinogens can alter the methylation status of specific genes. The rodent hepatocarcinogens trichloroethylene (TCE), dichloroacetic acid (DCA) and trichloroacetic acid (TCA) induce the liver mRNA and protein expression of the proto-oncogenes \textit{c-jun} and \textit{c-myc} within 100 min of exposure (Tao et al., 2000). This increase in expression is accompanied by demethylation of CpG dinucleotides in the regulatory regions of both genes following 5 days of exposure to these compounds. Taken together, these experiments clearly demonstrate that certain carcinogenic chemicals elicit alterations in DNA methylation, and that strain differences in the extent of these changes can reflect relative sensitivity to the carcinogenic agent. We do not mean to imply that all non-genotoxic carcinogens exert their effects by disrupting DNA methylation. Rather, it is possible that direct disruption of epigenetic status may be a key feature of the mechanism of action of some non-genotoxic carcinogens.

3.2. Metals

Toxic metals disrupt a wide range of cellular processes and bring about a variety of toxic effects. A hypothesis that is gaining in popularity is that the carcinogenic effects of some metals are mediated by global and targeted disruption of DNA methylation. Cadmium (Cd) causes DNA hypomethylation in a rat
liver cell line following exposure for 1 week, while exposure for 10 weeks induces hypermethylation of DNA (Takiguchi et al., 2003). Another carcinogenic metal, arsenic (As), elicits a genome-wide decrease in methylation in the rat liver cell line TRL1215 in a dose- and time-dependent fashion following chronic treatment (Zhao et al., 1997).

Although it has been established that some nickel (Ni) compounds are carcinogenic, the precise mechanism of Ni-induced tumour formation has not been defined, although a number of mechanisms have been proposed (reviewed by Cangul et al., 2002). As seen with Cd and As, Ni induces changes in DNA methylation that lead to alterations in gene expression. Ni exposure resulted in silencing of a bacterial gene that had been integrated into a transgenic cell line (Lee et al., 1995). This silencing was not caused by changes in gene sequence but, instead, was accompanied by increased DNA methylation, in the coding and flanking regions of the transgene, and formation of heterochromatin (a highly compacted state of chromatin that is associated with DNA hypermethylation and gene silencing).

4. How do chemicals and metals perturb DNA methylation and epigenetic status?

The evidence described above demonstrates that non-genotoxic chemical carcinogens and metals can perturb DNA methylation patterns and, therefore, the epigenetic status of a cell. To understand the molecular mechanism by which they achieve this, we must first consider the cellular machinery responsible for establishing and maintaining epigenetic information. DNA methylation is catalysed by a family of DNA methyltransferase enzymes (DNMTs). Three distinct enzymes exist in mammals: DNMT1, DNMT3A and DNMT3B (El-Osta, 2003). DNMT1 is often called a ‘maintenance’ methyltransferase, because its role appears to be to maintain DNA methylation patterns following gene expression. The other two enzymes are responsible for the de novo methylation of genomic DNA following implantation. The major functional consequence of DNA methylation is an alteration in the degree of compaction of the chromatin template within which the DNA is packaged. In simple terms, this compaction excludes the cellular transcriptional machinery and switches off gene expression (Orphanides and Reinberg, 2002). Chromatin compaction requires the concerted activities of at least four other classes of proteins (reviewed by Vaquero et al., 2003). These are: (1) DNA remodelling enzymes, which use energy from ATP hydrolysis to ‘remodel’ the structure of chromatin (Narlikar et al., 2002), (2) histone acetyltransferases (HATs) (Carrozza et al., 2003), (3) histone deacetylases (HDACs) (de Ruijter et al., 2003), which acetylate and deacetylate, respectively, lysine residues in the histone protein components of chromatin, (4) histone methyltransferases (HMTs), which methylate lysine or arginine residues in histones (Zhang and Reinberg, 2001). All four classes of enzyme, as well as the DNMTs, play crucial roles in the establishment and maintenance of DNA methylation patterns and epigenetic status. It is, therefore, possible that the carcinogenic chemicals and metals described above disrupt DNA methylation by inhibiting directly the activities of these enzymes.

What is the evidence for this? First, Cd inhibits the activity of a model bacterial DNMT in vitro, possibly by associating with the DNA binding domain of the enzyme (Takiguchi et al., 2003). In addition, Ni inhibits the activity of the Gcn5p HAT enzyme in vitro and causes a global decrease in the acetylation of specific lysine residues in histone H4 in vivo (Broday et al., 2000). Ni exposure also causes a reduction in histone H3 and H4 acetylation of a bacterial gene integrated into a transgenic cell line (Yan et al., 2003). Therefore, it appears that carcinogenic metals can inhibit directly enzymatic activities required for the maintenance and transmission of epigenetic information. It is possible that other carcinogens can also disrupt these activities. Precedents for the chemical perturbation of these enzymes can easily be found: DNMT and HDAC inhibitors, such as 5-aza-cytidine (Juttermann et al., 1994) and suberoylanilide hydroxamic acid (SAHA) (Kelly et al., 2003), respectively, induce alterations in DNA methylation and gene expression. Interestingly, these chemicals may be capable of reversing the DNA methylation changes found in tumours and, therefore, are showing promise as anti-tumour agents (Brown and Strathee, 2002; Kelly et al., 2003). As we discuss in the next section, xenobiotics may also perturb DNA methylation patterns by affecting the metabolic pathway that leads to the synthesis of S-adenosylmethionine (SAM), the major donor of
methyl groups in vivo. Intriguingly, the hypomethylation and induction of c-jun and c-myc induced by the hepatocarcinogens TCE, DCA and TCA is prevented by concomitant injection of methionine, suggesting that these compounds may induce hypomethylation by depleting cells of SAM (Tao et al., 2000).

5. Dietary modulation of DNA methylation and epigenetic status

It is well-established that diet, and nutrient status in general, affects significantly epigenetic programming in the liver and other organs (Huang, 2002). Insufficient dietary levels of methyl group donor molecules (e.g. amino acids, such as choline and methionine and vitamins, such as folate and vitamin B12) results in global genomic DNA hypomethylation, which can lead to adverse effects. Rats fed on a diet devoid of methyl-containing amino acids exhibit markedly lower hepatic levels of methylated cytosines than rats fed a control diet (Wilson et al., 1984). Moreover, diet-induced epigenetic alterations have been associated with cellular transformation: rats fed a choline-deficient diet exhibit a higher incidence of liver tumours (Locker et al., 1986). Similarly, transgenic mice with lower levels of the major physiological methyl donor, SAM, resulting from inactivation of the liver-specific methionine adenosyltransferase gene (MAT1A), develop a range of hepatic injuries (Lu et al., 2001). These studies establish that dietary deficiencies in amino acids involved in the synthesis of SAM decrease levels of DNA methylation and can result in adverse effects.

6. Perturbation of epigenetic status by estrogenic non-genotoxic carcinogens

Epigenetic mechanisms may also underpin the mechanism of action of certain classes of developmental toxicants. DNA methylation changes play a key role during mammalian embryonic development, a process that involves the tightly controlled temporal and spatial regulation of gene expression in order to establish the diversity of cell and tissue types that make up an adult mammal (Reik et al., 2001). Exposure to xenobiotics during critical periods of mammalian development can induce persistent and heritable changes in DNA methylation and, therefore, gene regulation, leading to adverse biological effects. The potential role of xenobiotic-induced epigenetic changes in developmental toxicity is exemplified by exposure to diethylstilbestrol (DES), a developmental stage-specific non-genotoxic carcinogen.

Millions of women were exposed to DES in utero between 1947 and 1971, when their mothers took the drug during pregnancy to prevent miscarriage (Laitman, 2002). In utero exposure of women to DES during the first 3 months of pregnancy resulted in a high incidence of abnormalities in reproductive tissues, subsequent infertility problems and an increased risk of developing clear cell adenocarcinoma of the vagina or cervix at a young age (Marselos and Tomatis, 1992). DES-induced carcinogenesis has also been modelled in rodents: perinatal DES exposure in mice induces reproductive tract abnormalities (Marselos and Tomatis, 1993). Furthermore, exposure to DES or the phytoestrogen genistein during postnatal days 1–5 (a point at which the development of the mouse uterus is roughly equivalent to that seen in humans at the end of the first trimester of pregnancy) resulted in an elevated incidence of uterine epithelial cancers in animals aged between 18 and 24 months (Newbold et al., 1990, 2001). In contrast, mice exposed to DES 20 days after birth did not show increased cancer incidence in later life.

What is the mechanism underlying this narrow developmental window of sensitivity to estrogenic xenobiotics such as DES? Studies of the effects of perinatal DES exposure in rodents support a mechanism whereby DNA methylation changes in the promoters of estrogen-responsive genes result in persistent alterations in gene expression and, therefore, the phenotype of exposed cells (reviewed by Li et al., 2003a). Exposure of neonatal mice to estrogens, including DES and genistein, results in the persistent up- or down-regulation of certain genes under the control of estrogen receptors (e.g. lactoferrin, EGF, c-fos, c-jun, c-myc (Nelson et al., 1994; Kamya et al., 1996; Yamashita et al., 2001; Falck and Fournier, 1996; Hoxa-10 and Hoxa-11 (Ma et al., 1998; Couse et al., 2001; Block et al., 2000)). Remarkably these altered gene expression patterns are sustained throughout development and into adulthood. This is in contrast to the transient regulation
of gene expression induced by estrogen exposure in prepubertal or ovariectomised adult mice.

At least two of the genes persistently up-regulated by neonatal DES exposure, lactoferrin (Li et al., 1997) and c-fos (Li et al., 2003b), also exhibit altered DNA methylation patterns, a phenomenon not seen in adult mice exposed to the same dose of DES. Importantly, in neonatally exposed mice, altered DNA methylation appears to require both the initial neonatal exposure to an estrogen and additional signals (most likely ovarian hormones) that arise during the onset of puberty (Li et al., 1997). The molecular mechanisms underlying the persistent changes in gene expression and DNA methylation induced by neonatal exposure to DES remain to be elucidated. However, it is interesting that epigenetic alterations are found in specific CpG sequences within or close to estrogen receptor-regulated genes. Neonatal exposure of male mice to estrogenic compounds also results in persistent gene expression changes in reproductive tissues (Salo et al., 1997; Adachi et al., 2002), although the DNA methylation status in these tissues has not been examined. Aberrant DNA methylation following prenatal DES exposure has also been reported at ribosomal DNA sequences (Alworth et al., 2002). An important question that remains to be answered is which epigenetic changes (and associated alterations in gene expression) predispose DES-exposed neonatal mice to cancer in later life.

7. Can chemically induced epigenetic alterations be transmitted between generations?

Experiments in mice suggest that the adverse effects associated with DES exposure in early development can be passed on to subsequent generations. The female offspring of female mice exposed prenatally or neonatally to DES exhibit an increased incidence of uterine tumours (Walker, 1984; Newbold et al., 1998). A similar increased cancer incidence has been observed in the progeny of male mice exposed prenatally to DES (Tursunov et al., 1992). It is possible that these trans-generational effects are mediated by the inheritance of stable, DES-induced alterations in DNA methylation at specific gene loci.

The possibility for stable inter-generational phenotypic change as a result of environmentally induced alterations in DNA methylation has been demonstrated using the viable yellow agouti (Ayv) mouse model. The genome of Ayv mice contains a regulatory insert in the agouti gene, which encodes a signalling molecule involved in hair pigmentation (described by Waterland and Jirtle, 2003). This insert (intracisternal A particle, IAP) can be silenced by CpG methylation. This results in a range of altered phenotypes, which vary according to the degree of DNA methylation at this locus. Hypomethylation of the locus results in expression of the agouti protein and causes yellow coat pigmentation and obesity. Conversely, hypermethylation inhibits agouti expression and is associated with brown coats and normal body weights. A recent study demonstrated that dietary supplementation with methyl group donors (choline, methionine, betaine, folic acid and vitamin B12) in nulliparous mice for a 2-week period prior to mating resulted in a higher frequency of lean, brown pups (Waterland and Jirtle, 2003). Strikingly, pups born from mothers fed a control diet remained fat and yellow. The observed pup phenotype corresponded to the level of diet-associated DNA methylation at the agouti locus. Therefore, dietary modulation of DNA methylation levels can lead to phenotypic change in offspring. The idea of inter-generational transmission of DNA methylation patterns goes against current models of mammalian development, which postulate that epigenetic marks are erased during fertilisation (Reik et al., 2001). However, it is possible that some epigenetic marks survive this process.

These experiments suggest that the ‘nutritional history’ of an organism can affect the phenotype of its offspring through the inheritance of epigenetic alterations. A recent study suggested that this phenomenon might also occur in human populations. Analysis of the grandchildren of a cohort of Swedish men demonstrated a correlation between the availability of food to the grandparent just before puberty and the disease incidence in their grandchildren (Kaastra et al., 2002; Dennis, 2003). The grandchildren of well-fed grandparents had a greater incidence of diabetes, while those related to under-fed grandparents had a lower incidence of heart disease. Further research is needed to establish whether environmental influences (such as nutrition) can result in long-lasting, trans-generational effects in animals and humans.
8. Summary and future opportunities

Although it has not yet been established that toxicant-induced changes in DNA methylation play a direct role in chemical carcinogenesis, there exists sufficient anecdotal and circumstantial evidence to suggest that the topic is worthy of future attention. In particular, the following areas should be addressed:

(1) The mechanism by which toxicants can (directly or indirectly) alter DNA methylation patterns and epigenetic status. It will be interesting to determine whether the activities of members the large family of SET domain-containing HMT enzymes identified recently (reviewed by Vaquero et al., 2003) can be affected by toxicants.

(2) Whether DNA methylation changes induced by carcinogens (and found in tumours) play causative roles in carcinogenesis or are merely a consequence of the transformed state.

(3) The role played by DNA methylation in normal physiological processes and adaptation to environmental change.

(4) The possibility that changes in DNA methylation can act as biomarkers of adverse effect (Watson et al., 2003). DNA methylation changes in human plasma show much promise for the early detection of cancer (Laird, 2003).

(5) The potential that some epigenetic marks may survive the general epigenetic reprogramming that is believed to occur during fertilisation, thereby allowing inter-generational transmission of epigenetic changes induced by environmental factors.

(6) The development and application of methods for locating sites of altered DNA methylation (Novik et al., 2002).

References


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