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Perturbation of epigenetic status by toxicants

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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).

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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

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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 *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 mobilising 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 *c-jun* and *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 DNA replication. 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 Strathdee, 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