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Review

A new paradigm in toxicology and teratology: Altering gene activity in the absence of DNA sequence variation☆

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Abstract

'Epigenetics' is a heritable phenomenon without change in primary DNA sequence. In recent years, this field has attracted much attention as more epigenetic controls of gene activities are being discovered. Such epigenetic controls ensue from an interplay of DNA methylation, histone modifications, and RNA-mediated pathways from non-coding RNAs, notably silencing RNA (siRNA) and microRNA (miRNA). Although epigenetic regulation is inherent to normal development and differentiation, this can be misdirected leading to a number of diseases including cancer. All the same, many of the processes can be reversed offering a hope for epigenetic therapies such as inhibitors of enzymes controlling epigenetic modifications, specifically DNA methyltransferases, histone deacetylases, and RNAi therapeutics. 'In utero' or early life exposures to dietary and environmental exposures can have a profound effect on our epigenetic code, the so-called 'epigenome', resulting in birth defects and diseases developed later in life. Indeed, examples are accumulating in which environmental exposures can be attributed to epigenetic causes, an encouraging edge towards greater understanding of the contribution of epigenetic influences of environmental exposures. Routine analysis of epigenetic modifications as part of the mechanisms of action of environmental contaminants is in order. There is, however, an explosion of research in the field of epigenetics and to keep abreast of these developments could be a challenge. In this paper, we provide an overview of epigenetic mechanisms focusing on recent reviews and studies to serve as an entry point into the realm of 'environmental epigenetics'.

Keywords: Epigenetics; DNA methylation; Histone modifications; Chromatin remodelling; RNA silencing pathways; Genomic imprinting; Genomic reprogramming

Contents

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1. Introduction

Regulation of gene expression is a crucial process from early development to adulthood. It is not only important when a gene is turned 'on' or 'off', but variation in gene expressions can lead to phenotypic diversity. The factors that contribute exactly to the regulation of gene expression are still not fully understood, but emerging knowledge shows that this process is not only governed by the genetic make-up of an individual, but also by epigenetic factors. Indeed, growing evidence points to an additional epigenetic code, notably the 'epigenome'. Nonetheless, the extent by which epigenetic heterogeneity at the level of cell type, tissue, and organ within an individual as well as among individuals plays a role still needs to be determined [1].

The term 'epigenetics', was introduced by the developmental biologist Conrad H. Waddington (1905–1975), in 1942 to describe interactions of genes and their environment [2,3]. Epigenetics is now used to describe heritable changes in gene expression that are not coded in the DNA sequence, but an interplay of DNA methylation, histone modifications and expression of non-coding RNAs, in the regulation of gene expression patterns. Epigenetic regulation is not only important for generating diversity of cell types during mammalian development, but also in maintaining the stability and integrity of the expression profiles of different cell types. Although epigenetic processes are essential for development and differentiation, they can become misdirected leading to birth malformations and various human diseases, especially cancer. This review aims to provide an overview on epigenetic mechanisms on gene expression, their role in normal development and disease, and relevance of epigenetic phenomena in toxicology and teratology.

2. Epigenetic mechanisms: it is not all in the DNA

Gene regulation without sequence alterations is brought about by epigenetic mechanisms that involve DNA methylation, histone modifications, and RNA-associated pathways. These three epigenetic regulatory mechanisms are generally associated with the initiation and maintenance of silencing of gene expressions, and interact to each other to effect heritable silencing [4]. An upsurge of research activities is evident and more epigenetic mechanisms are newly discovered. For instance, the phenomena in which alterations in gene activity can also be induced by direct interactions between chromosomal regions that are positioned at long-distances from one another, resulting in activation or repression [5]. Furthermore, at a recent Keystone symposia on stem cell biology, evidence was presented for epigenetic regulation of stem cell fate which obviously is an exciting area of research and offers promising applications in cell-based therapies.

2.1. DNA methylation

DNA methylation has long been recognized as an epigenetic silencing mechanism of fundamental importance in embryonic development, transcription, chromatin structure, X-chromosome inactivation, genomic imprinting, and chromosome instability. Modification by DNA methylation occurs by the covalent addition of a methyl group to position 5 of the cytosine ring, creating 5-methylcytosine [6,7]. Basically, CpG dinucleotides are the sites of almost all methylation in mammals. CpG dinucleotides are not equally distributed throughout the genome; instead, they occur in clusters of either large repetitive sequences (such as rDNA, satellite sequences or centromeric repeats) or in short CG-rich DNA stretches, known as CpG islands (CGIs), often found in the promoter region and the first exon of genes. Generally, a CpG island is defined as a contiguous window of DNA of at least 200 base pairs in which the GC content is at least 50% and the observed/expected ratio for the occurrence of CpG > 0.6. Recently, a more stringent definition has been proposed in which a CpG island as a 500 base- pair-window with a GC content of at least 55% and an observed/expected CpG frequency of at least 0.65% to exclude most Alu repeat sequences. Whereas CGIs are usually unmethylated in healthy tissues, repetitive sequences are highly methylated. However, of the CGIs that were hypermethylated, none had a CpG density greater than 10%. Recently, it has been shown that DNA methylation can affect transcription of genes whose 5′ UTRs had low CpG density ('non-CGI promoters') [8].

The mammalian DNA methylation machinery is mediated by the DNA methyltransferases (DNMTs), which establish and maintain DNA methylation patterns; and the methyl-CpG binding proteins (MBDs), which are involved in 'reading'

methylation marks [7]. DNMT1 is the main enzyme in mammals and preferentially recognizes hemimethylated DNA during replication and thus re-establishes the original methylation patterns after cell divisions, referred to as maintenance methylation. Maintenance of DNA methylation by DNMT1 is crucial for embryonic development, but DNMT1 is also the same enzyme required for faithfully maintaining DNA methylation patterns in human cancer cells and is essential for their proliferation and survival [9]. In contrast, the *de novo* methyltransferases DNMT3a and DNMT3b target new unmethylated DNA sites. Methyl-CpG binding proteins contain the conserved DNA binding motif methyl-cytosine binding domain, which preferentially binds to methylated CpG dinucleotides. These proteins serve as transcriptional repressors, mediating gene silencing via DNA cytosine methylation.

DNA methylation influences transcription, in which the methyl group that protrudes from the cytosine nucleotide into the major groove of the DNA, displaces transcription factors that normally bind to the DNA, or attracts methyl-binding domains, which in turn are associated with gene silencing and chromatin compaction [10]. DNA methylation aberrations can occur as either hypo- or hypermethylation. Both forms can lead to chromosomal instability and transcriptional gene silencing, and both have been implicated in a variety of human malignancies. Aberrant DNA methylation can lead to a number of human diseases [7].

A number of detection techniques are available to study methylation patterns, which are basically based on enzymatic hydrolysis, digestion with methylation-sensitive restriction enzymes, or bisulfite treatment of genomic DNA prior to downstream analysis [6,11,12]. These techniques can be used in mapping DNA methylation on individual gene sequences or to detect DNA methylation genome-wide, but each has its limitations. Recently, a further global methylation approach was demonstrated, i.e. a methyl-DNA immunoprecipitation (mDIP) assay that uses antibodies specific for 5-methyl-cytosine residues [13]. Most applications are based on bisulfite treatment of genomic DNA, which converts cytosine to uracil but methylated cytosines remain unaltered in this process. After PCR amplification, uracil will be converted to thymidine, which will be determined by direct PCR sequencing (bisulfite sequencing) or methylation-specific PCR (MSP-PCR). In bisulfite sequencing, primers are designed not to contain any CpGs to avoid discrimination against methylated or unmethylated DNA. In MSP-PCR, two pairs of primers are designed; one is specific for (M) methylated DNA and the other for (U) unmethylated DNA. After bisulfite-sequencing or M-specific PCR, amplified fragments are usually cloned to determine the degree of methylation.

2.2. Histone modifications

After DNA methylation, a second epigenetic control of gene expression involves the proteins (histones) that package DNA into chromatin. These proteins, called histones, determine whether the chromatin is tightly packed, in which case gene expression is shut down (or silenced), or relaxed, in which case gene expression is active. Essentially, chromatin comprises DNA, histones H1, H2A, H2B, H3, and H4, and non-histone proteins [14,15]. DNA and histones form repetitive nucleoprotein units, the nucleosomal core particles. Each particle consists of 146 bp of DNA wrapped around an octamer of core histones (1 H3-H3-H4-H4 tetramer and 2 H2A-H2B dimers). The DNA located between nucleosomal core particles is associated with histone H1. This 11 nm histone fiber is then further packed into an irregular 30 nm chromatin fiber structure, which is coiled into even more complex structures to eventually assemble the chromosome [16]. Each core histone is composed of a structured domain and an unstructured amino-terminal 'tail' of 25–40 residues. The amino terminal tails of histones protrude from the nucleosomal surface. Covalent modifications of these tails affect the structure of chromatin and form the basis for the epigenetic regulation of chromatin structure and gene function. Amino acid residues in histone tails are modified by covalent acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and biotinylation among others to regulate gene transcription, mitotic condensation of chromatin, and DNA repair [14,17–19]. Furthermore, there is cross-talk between histone modifications [15], in line with the histone code hypothesis which states that specific combinations of histone modifications specify the structural state of chromatin [20]. Effector proteins read and carry out the code's instructions to specify heterochromatin formation, DNA that is tightly packed with nucleosomes, or more loosely packed euchromatin. Compared with euchromatin, heterochromatic DNA is largely inaccessible to transcription factors and chromatin remodelers, making it relatively transcriptionally inert. Specifically, combinations of histone modifications at the N-terminal regions are reversible, and that protein binding to these tails and different histone protein associations bring about a variety of functional outcomes. Thus, defining the histone code as part of the 'epigenetic code' to result in cellular memory will be the challenge of future research. Nonetheless, the concept of a heritable epigenetic code based on histone modifications is still the subject of a controversy. The evidence available so far remains inconclusive.

2.2.1. Histone acetylation/deacetylation

Histone acetylation is a reaction where an acetyl group is introduced usually to lysine residues at the N terminus of histone protein. The opposite reaction is histone deacetylation, i.e. the removal of acetyl group. The highly conserved histone H3 lysines at amino-terminal amino-acid positions 9, 14, 18 and 23, and H4 lysines 5, 8, 12 and 16 are frequently targeted for modification. The source of the acetyl group in histone acetylation is acetyl-coenzyme A, and in histone deacetylation the acetyl group is transferred to coenzyme A. Histone acetylation is catalyzed by histone acetyltransferases (HATs), while histone deacetylation is catalyzed by histone deacetylases (denoted by HDs or HDACs). Several different forms of HATs and HDACs have been identified. For instance, there are three major classes of mammalian HDACs: Rpd3 (class 1), Hda1 (class II), and Sir2 (class III). Acetylation removes positive charges thereby reducing the affinity between histones and DNA. Thus, in most cases, histone acetylation enhances transcription while histone