Mutation Research xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Epigenetic profiles as defined signatures of xenobiotic exposure

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ARTICLE INFO

Article history:
Received 23 August 2013
Accepted 24 August 2013
Available online xxx

Keywords:
Epigenetics
chemical safety
5hmC
5-hydroxymethylcytosine
5mC
5-methylcytosine
DNA methylation
cancer

ABSTRACT

With the advent of high resolution sequencing technologies there has been increasing interest in the study of genome-wide epigenetic modification patterns that govern the underlying gene expression events of a particular cell or tissue type. There is now mounting evidence that perturbations to the epigenetic landscape occur during a host of cellular processes including normal proliferation/differentiation and aberrant outcomes such as carcinogenesis. Furthermore, epigenetic perturbations have been associated with exposure to a range of drugs and toxicants, including non-genotoxic carcinogens (NGCs). Although a variety of epigenetic modifications induced by NGCs have been studied previously, recent genome-wide integrated epigenomic and transcriptomic studies reveal for the first time the extent and dynamic nature of the epigenetic perturbations resulting from xenobiotic exposure. The interrogation and integration of one such epigenetic mark, the newly discovered 5-hydroxymethylcytosine (5hmC) modification, reveals that drug treatment associated perturbations of the epigenome can result in unique epigenetic signatures. This review focuses on how recent advances in the field of epigenetics can enhance our mechanistic understanding of xenobiotic exposure and provide novel safety biomarkers.

1. Introduction

The term "Epigenetics" is typically described as the phenomenon whereby changes in gene expression occur without direct changes to DNA sequence itself [1]. These changes are typically heritable being passed on through cell division through the generations. The name, derived from the Greek "epi" meaning "over" or "above" describes the situation where the DNA sequence can be regulated outside of its primary sequence. This control is brought about both by the direct chemical modification of the DNA itself along with its packaging into chromatin, which is linked to the modification state of the core histone protein complex around which the DNA is wrapped; the nucleosome. Epigenetic control is particularly prominent during development, e.g. as pluripotent embryonic stem cells (ESC) containing the same DNA sequences (barring accumulated mutations) that differentiate into many functionally diverse cell types, each of which must express a different panel of genes for functionality [2]. As such these epigenetic patterns must be unique to a particular cell type as they define the associated transcriptome. Over the past decade the importance of such epigenetic regulation has become apparent in a range of developmental disorders and diseases [3,4] as well as playing critical roles during carcinogenesis [5–7]. Furthermore, changes to the normal epigenetic patterns are believed to be important for the adverse effects associated with exposure to some drugs and toxicants (e.g. diethylstilbestrol, tamoxifen and arsenic are all known to exhibit changes to normal DNA methylation and histone modification patterns [8–10]) specifically those belonging to the class of non-genotoxic carcinogens (NGCs) including phenobarbital and peroxisome proliferators [11–18]. The study of NGC exposure in preclinical animal models is of particular interest as the associated progression to carcinogenesis is believed to occur without an initial change to the underlying DNA sequence ("non-genotoxic") and is therefore a useful model to investigate early perturbations to the epigenome following xenobiotic exposure. In turn these early epigenetic perturbations are postulated to create an epigenetic environment that is predisposed to a spectrum of cancer-causing mutations, analogous to the epigenetic progenitor model for human cancer [7]. Here we present an overview of recent work

Abbreviations: 5caC, 5-carboxylcytosine; 5fC, 5-formylcytosine; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; CAR, constitutive androstane receptor; CGI, CpG island; CpG, cytosinephosophate-guanine; CNV, copy number variation; DEN, diethylnitrosamine; DNMT, DNA methyltransferases; ESC, embryonic stem cell; MBD, methylated binding domain; MeDIP, methyl DNA immunoprecipitation; NGC, non-genotoxic carcinogen; PB, phenobarbital; RAM, region of altered methylation; RNAPII, RNA polymerase II; TDG, thymidine glycosylase; TET, ten-eleven-translocation; TF, transcription factor; TSS, transcriptional start site.

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[1] Member of the BioMARkers & molecular tumour classification for non-genotoxic CARcinogenesis (MARCAR) consortium (www.imi-marcar.eu).

[2] Supported by the MARCAR project.

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http://dx.doi.org/10.1016/j.mrgentox.2013.08.007

that characterises drug-induced perturbations of newly discovered epigenetic modifications. We discuss the likely functional consequences of these epigenetic perturbations and their potential utility for enhancing safety assessment.

1.1. DNA modification status is integral to the regulation of higher order chromatin

Epigenetic marks can be grouped into those which are either deposited directly onto the DNA or those which mark the N-terminal tails of the associated histone proteins. The wrapping of DNA around these histone proteins forms an octameric protein structure called the nucleosome and the subsequent compaction of these structures packages the DNA into the nucleus of a cell [19]. It is this higher order structure that is important in the regulation of gene expression events with an "open" or euchromatic state able to facilitate transcription whilst a compact or heterochromatic state is associated with silenced regions of the genome [20–22].

Mammalian DNA is typically modified on the 5th carbon of a cytosine base in a cytosine–phosphate–guanine (CpG) dinucleotide sequence [2,23]. It is thought that ~4% of all cytosines (~80% of all the CpG dinucleotides) are modified by the incorporation of a methyl group resulting in the formation of 5-methylcytosine (5mC) [24]. DNA marked in such a way is typically found to be associated with repetitive DNA sequences and promoters rich in methylated CpGs are thought to be transcriptionally silenced [25]. Such regions of methylated CpG sequence are then thought to be bound by proteins containing methylated binding domains (MBDs) which recruit in further chromatin modifying proteins which results in the formation of a silent and compact chromatin environment [26–28]. Although the majority of CpG dinucleotides are found modified in such a way, a functionally significant fraction exists which is both non-modified and typically promoter associated [29]. Such regions – termed CpG islands (CGIs) – are thought to result in the formation of a transcriptionally permissive chromatin environment through the attraction of a second set of proteins which contain a conserved CXXC domain specific to the non-modified CpG motif [30]. Therefore the regulation of the DNA modification state is a critical factor affecting the higher order chromatin state, which in turn affects the transcriptome of a particular cell or tissue.

The heritable 5mC patterns are set up both de novo as well as copied over onto newly synthesised strands of DNA upon replication by a group of proteins called the DNA methyltransferases (DNMTs). Although the maintenance of the 5mC mark is well studied, the pathways governing active removal of the resulting 5mC marks are only now beginning to be understood due to the recent discovery of further modified forms of cytosine nucleotides. The first of these newly discovered modifications was 5-hydroxymethylcytosine (5hmC), a modification which was initially discovered over 35 years earlier [31,32]. Recent work reveals that this mark is less abundant than that of 5mC, with between 0.1 and 0.7% of all cytosines marked by hydroxymethylation [33]. In parallel to this work it was shown that a group of enzymes belonging to the TET family (1–3) of iron and α-ketoglutarate dependent dioxygenases utilise molecular oxygen to transfer a hydroxyl group to 5mC to form 5hmC [34–38]. These TET proteins are frequently mutated or inhibited in many human acute myeloid leukaemias [39,40] as well as substantially reduced in their expression levels in many human cancers and cell lines [41,42]. Since this early work there has been a large degree of interest in the study of 5hmC marked DNA with several groups mapping the distribution of the mark in both cultured cells as well as in several tissue types [11,34,42–52]. A consensus view is that 5hmC modified CpGs are typically found to be highly enriched in the bodies of actively transcribing genes and are present to some degree at enhancer elements and a small cohort of regions spanning an annotated transcriptional start site (TSS) [11,45,53]. Using tiled arrays, Nestor et al. recently revealed that 5hmC patterns are highly similar within but not across tissue types [42] highlighting that fact that 5hmC can be used as an identifier of cell or tissue type. In the mouse brain, global 5hmC patterns were seen to change dynamically with age [54], whilst recent work has found that during melanoma formation there is a global loss of 5hmC, indicating that the 5hmC modification may also be useful as an identifier of disease state [55].

In addition to 5hmC, a further two forms of cytosine modification have also been recently characterised; those of 5-formyl cytosine (5fC) and 5-carboxylcytosine (5caC). These two marks were shown to be sequentially produced from 5mC upon the overexpression of the TET enzymes setting up a potential demethylation pathway driven by the TET proteins (Fig. 1) [56]. Studies thus far reveal that the levels of these two modifications are incredibly low ($\sim 20 \times 10^{-6}$% cytosine for 5fC and 3×10^{-6}% in ES cells) and as such may represent transient and ongoing DNA demethylation. However recent work from the He and Zhang labs find that both 5caC and 5fC are found enriched over the bodies of actively transcribing genes as well as over "poised" enhancer regions (H3K4me1 + ve, H3K27ac −ve marked enhancers [57–59]).

As relatively little is known regarding the normal distributions and functional roles associated with these recently discovered DNA modifications it will be important firstly to properly characterise the marks further, before attempting to study such modifications in the context of mechanistic toxicology studies. Nevertheless, the ever expanding number of potential epigenetic modifications available for mechanistic investigations of gene regulation will ultimately result in highly detailed combined datasets and therefore lead to a deeper understanding of unique xenobiotic-induced epigenetic signatures.

1.2. Enhancing mechanistic toxicology through the incorporation of epigenetics

The potential impact of epigenomics on drug safety sciences has recently been extensively reviewed [14,60]. In brief, traditional toxicity testing has focussed on the phenotypic outcomes of drug administration which would result in a change in the behaviour of an animal or the production of a tumour without a full understanding of the mechanism of drug action. Genotoxicity testing protocols focus on the characterisation of genetic alterations which arise through chemical exposure such as sequence mutation of genes involved in tumorigenesis or the alteration of chromosomal number (aneuploidy or polyploidy) and copy number variation (CNV). Such genetic tests are often insufficient to accurately predict the progression of rodent carcinogenesis and are typically poor at discriminating genotoxic from non-genotoxic agents [61]. With the field of epigenetic research advancing at an accelerated rate alongside technologies that allow high-resolution genome-wide characterisation of multiple epigenetic marks, there are now many new opportunities for enhancing mechanistic toxicology investigations at the gene regulation level. Since epigenetic perturbations can lead to both short-term and longer-lasting changes in gene expression it is highly probable that integrated epigenomic and transcriptomic studies will reveal valuable information about xenobiotic-induced mechanisms of toxicity at very early stages of exposure as well as facilitating the prediction of longer-term toxicities such as carcinogenesis. At present, epigenetic endpoints are likely to be most useful for enhancing mechanistic investigations. Several knowledge gaps need to be filled prior to the routine incorporation of epigenetic endpoints into toxicity testing strategies.

Please cite this article in press as: J.P. Thomson, et al., Epigenetic profiles as defined signatures of xenobiotic exposure, Mutat. Res.: Genet. Toxicol. Environ. Mutagen. (2013), http://dx.doi.org/10.1016/j.mrgentox.2013.08.007