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Review

Importance of investigating epigenetic alterations for industry and regulators: An appraisal of current efforts by the Health and Environmental Sciences Institute

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ABSTRACT

Recent technological advances have led to rapid progress in the characterization of epigenetic modifications that control gene expression in a generally heritable way, and are likely involved in defining cellular phenotypes, developmental stages and disease status from one generation to the next. On November 18, 2013, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) held a symposium entitled "Advances in Assessing Adverse Epigenetic Effects of Drugs and Chemicals" in Washington, D.C. The goal of the symposium was to identify gaps in knowledge and highlight promising areas of progress that represent opportunities to utilize epigenomic profiling for risk assessment of drugs and chemicals. Epigenomic profiling has the potential to provide mechanistic information in toxicological safety assessments; this is especially relevant for the evaluation of carcinogenic or teratogenic potential and also for drugs that directly target epigenetic modifiers, like DNA methyltransferases or histone modifying enzymes. Furthermore, it can serve as an endpoint or marker for hazard characterization in chemical safety assessment. The assessment of epigenetic effects may also be approached with new model systems that could directly assess transgenerational effects or potentially sensitive stem cell populations. These would enhance the range of safety assessment tools for evaluating xenobiotics that perturb the epigenome. Here we provide a brief synopsis of the symposium, update findings since that time and then highlight potential directions for future collaborative efforts to incorporate epigenetic profiling into risk assessment.

Abbreviations: 5-caC, 5-carboxylcytosine; 5-fC, 5-formylcytosine; 5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; DNMT, DNA methyltransferase; EWAS, Epigenome-Wide Association Study; HDAC, histone deacetylase; hESC, human embryonic stem cell; iPSC, induced pluripotent stem cell; NOAEL, no-observed-adverse-effect level; PGC, primordial germ cells; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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Contents

1. Introduction

The term “epigenetics” has undergone much reinterpretation since first coined by Conrad Waddington, who proposed that development and evolution can be viewed as a succession of relatively stable states, separated by stages of instability and change (Stern, 2000; Waddington, 1940). A modern molecular view of epigenetics refers to the induction of stable changes in gene expression and chromatin organization that are independent of changes in the DNA sequence and propagated through cell division (Herceg et al., 2013). Key components involved in epigenetic mechanisms include DNA methylation, modifications of histone proteins and expression of non-coding RNA species, as well as X-chromosome inactivation and genomic imprinting which might be considered as secondary effects (Ferguson-Smith, 2011).

The fundamental structural unit within the chromatin structure is the nucleosome, which consists of 146 bp of DNA wrapped around a nucleosome core that is composed of evolutionary conserved histone proteins. The subsequent compaction of nucleosomes packages the DNA in the cell nucleus into characteristic cytological structures that include heterochromatin (Kouzarides, 2007). The higher order structure of chromatin is important in the regulation of gene expression with an “open” or euchromatic state able to facilitate transcription while a compact or heterochromatic state is associated with silenced regions of the genome (Sproul et al., 2005). Core histones (H2A, H2B, H3, and H4) have flexible N-terminal domains that contain residues that can be post-translationally modified at specific sites by methylation, acetylation, phosphorylation, ubiquitination, and sumoylation (Kouzarides, 2007). Sets of modifications are associated with transcriptionally active and silent states. Complex interactions between histones, modifying enzymes, DNA sequences, and other partner proteins contribute to gene expression regulation in specific contexts.

Epigenetic modifications can in general be grouped into those which are either deposited directly onto the DNA or those which mark the N-terminal tails of histone proteins. They are part of a complex network of interactions that fine-tune cells to their environmental conditions. These changes modify the DNA landscape to qualitatively and quantitatively determine how proteins interact with DNA segments, and thereby regulate gene expression globally and locally. Methylation catalyzed by DNA methyltransferases at the 5th position of cytosine residues in the context of the CpG dinucleotide results in formation of 5-methylcytosine (5-mC), which decorates the DNA landscape of mammalian somatic cells (Cruickshanks et al., 2013; Ehrlich et al., 1982; Jeltsch and Jurkowska, 2014). Methylation of cytosine at regulatory regions (promoters and enhancers) is associated with transcriptional repression and is considered to be rather stable in the genome. Although the majority of CpG dinucleotides are methylated in somatic DNA, a significant fraction of them, termed CpG islands (CGIs), are non-modified and are typically promoter associated (Illingworth et al., 2010).

The pathways governing active removal of 5-mC are only now beginning to be understood due to the recent discovery of further modified forms of cytosine nucleotides (Ito et al., 2011; Kriaucionis and Heintz, 2009). In 2009 the dioxygenase enzymes that convert 5-mC to 5-hydroxymethylcytosine (5-hmC), 5-formyl cytosine (5-fC) and 5-carboxylcytosine (5-caC) were described (Tahiliani et al., 2009). In terms of abundance 5-hmC is characteristically present at 10% or less relative to 5-mC, with 5-fC and 5-caC many orders of magnitude lower (Pfaffeneder et al., 2014). The attraction of studying 5-hmC in conjunction with 5-mC is that it is associated with reprogramming of DNA methylation patterns and is correlated with active genic regions in multiple tissues (Ficz et al., 2011; Nestor et al., 2012; Szulwach et al., 2011; Wu and Zhang, 2014). As such, 5-hmC profiles are indicative of cellular state (Laird et al., 2013).

In recent decades, the potential for xenobiotics to alter expression of genes has been well-studied. Termed toxicogenomics, this field endeavors to elucidate molecular mechanisms that underlie adverse responses to toxic agents by measuring alterations in messenger RNA (mRNA) expression. Such perturbations may then lead to altered protein expression and activity, thereby propagating the intracellular response to the agent. Recently attention has focused on understanding alterations of epigenetic modification in gene regulatory regions that control expression of genes. In that context, large epigenomic data sets for multiple tissues and disease states have been generated over the last decade that identify characteristic epigenetic alterations in cellular state, development, disease and cancer (Sproul and Meehan, 2013) (Fig. 1). Accumulating evidence suggests that epigenetic markers and/or the molecular machinery regulating them may be perturbed by exposure to various environmental, chemical, and