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Context is King — Questioning the causal role of DNA methylation in environmentally induced changes in gene expression

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Abstract

Across the field of developmental programming, changes in epigenetic modifications are often cited as the sole explanation for disease phenotype. However, there is now evidence that many of these changes should be viewed as a consequence of transcription and not the main driver of altered gene expression. Using a range of lifestyle and environmental exposures, this review will contrast instances where induced changes in DNA methylation can be inferred to be causal in the alteration of gene transcription, as compared with those that are either symptomatic or not biologically significant. Through this review, we find that most functionally significant changes map to gene enhancers and that low-magnitude changes cannot automatically be inferred to associate with changes in gene expression, especially as it pertains to the regulation of imprinted loci. Providing functional context and distinguishing between symptom and cause is essential for researchers in the field of developmental programming to transition beyond an inferred association and obtain a deeper, mechanistic understanding of the role epigenetic mechanisms have in altering the developmental program.

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Epigenetics, DNA methylation, Environmental exposure, Imprinted genes, Smoking, Fetal alcohol spectrum disorders, Flame retardants.

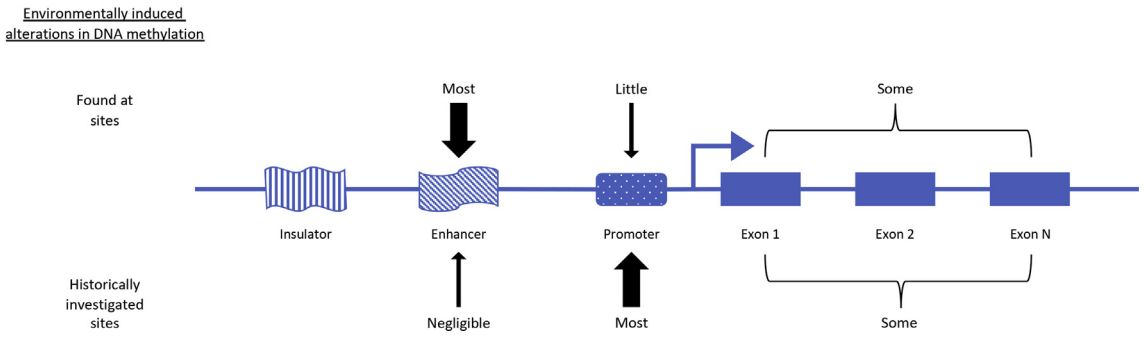
1. Introduction

Early work examining the regulation of imprinted genes, the suppression of transposable elements, and the process of X-chromosome inactivation identified a causal role of DNA methylation in transcriptional silencing [1–3]. Subsequent studies examining embryos produced through *in vitro* culture and/or somatic cell nuclear transfer revealed an association between environmentally induced changes in DNA methylation and the development of both placental and fetal growth defects [4,5]. As a consequence, DNA methylation was presumed to regulate protein-coding genes and a causal role applied to all instances of environmentally induced transcriptional change. However, as genome-wide resolution was gained, this overly simplistic view was disproven. For example, although levels of DNA methylation within the CpG islands located in imprint control regions (ICRs) and a few germline-specific genes directly associate with the suppression of transcription, there is no reasonable proof that this paradigm can be broadly extended to the promoter regions of all protein-coding genes, which are almost exclusively unmethylated in all tissue types regardless of expression status [6]. Furthermore, DNA methylation is enriched in the bodies of highly expressed genes where it suppresses spurious transcription but is not overtly repressive to the process of transcription [7]. Finally, work examining ligand-induced changes in gene expression reveal that gene-centered alterations in DNA methylation are secondary effects that follow transcriptional change, in some instances days later, and therefore, not causal in the induction of this change [8]. Using a range of environmental and lifestyle exposures, this review will distinguish environmentally induced changes in DNA methylation that are causal in facilitating altered patterns of transcription versus those that appear to arise as symptoms of environmental exposures and that may, in select instances, serve as biomarkers of past exposures (Fig. 1).

2. Smoking and alcohol: symptomatic changes in DNA methylation versus random noise?

The field of epigenetic research seeks to define the biochemical mechanisms by which the memory of environmental stimuli transmit through both cell division and potentially, onto subsequent generations [9]. As such, it is anticipated that epigenetic alterations to chromatin structure induced by environmental exposures should consistently overlap with

Fig. 1



Schematic overview of gene organization and common sites of environmentally induced changes in DNA methylation. Most studies of the environmental influence on the epigenome have focused on promoter CpG methylation. However, genome-wide bisulfite sequencing and other methylation analyses have revealed that a significant proportion of alterations occur elsewhere, including at intergenic regulatory regions such as enhancers and insulators.

changes in gene expression involved in the development of induced phenotypes. In line with this notion, studies examining the consequences of maternal smoking have identified a small cohort of genes that are now being implemented as biomarkers of sustained exposure during pregnancy [10–12]. Here, genes involved in detoxification, including those linked to the cytochrome P450 superfamily of enzymes (CYP1A1, CYP1B1) and components of the aryl hydrocarbon receptor pathway consistently display alterations in both gene expression and patterns of DNA methylation. Importantly, these same genes can be found in both adult smokers and individuals exposed during gestation [11,13,14]. However, elegant studies contrasting changes in both gene expression and DNA methylation between sets of identical twins after the cessation of smoking reveal that the observed changes in DNA methylation are not causal in imparting the observed alterations in gene expression but are themselves symptoms of exposure [14]. Specifically, smoking has a long-lasting influence on DNA methylation levels that persist after smoking cessation, while in contrast, changes in gene expression quickly revert. These data indicate that changes in DNA methylation represent a reliable biomarker of past exposure but are not themselves indicative of current patterns of gene expression or a viable explanation for the observed transcriptional changes. Consistent with this notion, this study was only able to identify a low ($r \sim 0.3$) correlation between DNA methylation and gene expression [14], which is consistent with the earliest genome-wide studies examining this epigenetic modification [15].

In contrast to work describing changes associated with smoking during pregnancy, studies examining alterations in DNA methylation induced by prenatal alcohol exposure, as well as in children with fetal alcohol spectrum disorder (FASD), have been varied and inconsistent [16–21]. For example, Portales-Casamar et al. [17] identified a rather large number of differentially methylated regions in individuals with FASD. Subsequently, Lussier et al. [18] bolstered this data set with additional samples and then went a step further by generating an algorithm using DNA methylation differences in one case–control cohort to predict FASD outcomes in another. Then, Sharp et al. [19] added their samples to this data set and came to the conclusion that no consistently differentially methylated regions could be identified. The inconsistencies in DNA methylation profiles observed in these studies is likely reflective of the wide variation observed in FASD phenotypes. To address this point, Cobben et al. [21] recently undertook a novel approach and stratified their patient cohort according to the presentation of phenotype. Specifically, they binned patients into growth-affected, central nervous system–affected and craniofacial-affected categories, which comprise the core FASD diagnosis [21]. However, less than 1% of the changes identified in this study were common with the previous bodies of work described above. As such, the authors concluded that none of the loci identified to date could reliably be applied in diagnostic or prognostic clinical practice [21]. In sum, these results are similar to work reported in adult studies of patients with alcohol use disorder, as well as gestational studies of DNA methylation using rodent models, which all report distinct sets of low-magnitude changes, variable cohorts of genes, and inconsistent directional changes [22–25]. Although there has been some suggestion that low-magnitude, environmentally induced changes in DNA methylation may be biologically significant [26], the lack of consistency identified in the substantial body of literature discussed previously suggests this does not apply to studies of FASDs. As mentioned, this may be due to the wide range of clinical phenotypes observed in this patient cohort or that alcohol does not appreciably impact the DNA methylome.

3. Low-magnitude changes at ICRs

Monoallelically expressed imprinted genes are controlled by parent-of-origin-specific patterns of DNA methylation that are stable throughout life and consistent across all three germ layers. Furthermore, the patterns of DNA methylation within most ICRs are fairly well characterized and their dysregulation is known to have adverse effects on growth and metabolism [27]. As such, imprinted genes are often used as the canary in a coal mine to study the effects of environmental stressors on DNA methylation. For example, suboptimal embryo culture, superovulation, and prenatal exposure to bisphenol A are all able to induce significant changes (in excess of 20%) in patterns of DNA methylation, which disrupt monoallelic patterns of gene expression and alter offspring growth [5,28,29]. However, many studies examining the impact of environmental toxicants on the regulation of imprinted genes report very modest changes in DNA methylation, often with only one CpG reaching statistical significance, and then automatically presume an impact on imprinted gene expression [26,30,31]. For example, studies examining the impact of smoking on the imprinted insulin-like growth factor II (IGF2) gene in umbilical cord blood reported that for every 1% change in DNA methylation at the IGF2 ICR, there was a doubling of IGF2 transcription [26,30]. This assertion runs contrary to numerous reports characterizing epigenetic mechanisms regulating imprinted gene expression both *in vitro* and *in vivo*. Clinical studies examining human buccal epithelial cells taken from normal healthy controls have found ~15% variation in the levels of DNA methylation across most imprint control centers [32], and *in vitro* studies examining environmentally induced changes in ICR methylation have found that a threshold decrease of 20% across the entire locus was needed to observe any impact on monoallelic gene expression [33]. This threshold change is consistent with the errors in imprinted gene expression induced by assisted reproductive technologies and exposure to high doses of bisphenol-A [28,29]. Therefore, caution should be infused into the interpretation of low-magnitude changes in DNA methylation on the regulation of imprinted genes [26,30]. It is also important to note that alterations in the expression of imprinted genes can be induced by toxicant exposures, prenatal alcohol exposure as an example, with no changes in DNA methylation, and where appropriate control of the silent allele is maintained [34].

4. DNA methylation: heritable memory of exposure or shared physiological response?

Time course studies examining ligand-induced changes in gene expression indicate that alterations in DNA methylation follow the induction of targeted loci [8]. Recent research has revealed that select flame retardants have the capacity to act as agonists or antagonists for a range of receptors, including those in the estrogen, thyroid hormone and peroxisome proliferator-activated receptor gamma (PPARγ) pathways [35–37]. Consistent with these effects, exposure to these toxicants is able to modify the DNA methylation profiles of the responsive genes. For example, two recent studies investigating the capacity of brominated flame retardants tetrabromodiphenyl ether (BDE-47) and pentabromodiphenyl ether (PBDE-99) to act as obesogens found that these toxicants were able to induce PPARγ, which was associated with a ~20% decrease in methylation at the PPARγ promoter [36,37]. Therefore, when investigating the effects of toxicants as potential endocrine disruptors, it is becoming more obvious that alterations in DNA methylation should be viewed as a consequence and not the main drivers of the outcome.

Given the correlation with transcriptional change, care should be taken to not automatically infer a role for DNA methylation in the transmission of an epigenetic memory of exposure. For example, identification of a common DNA methylation signature between mother–offspring pairs at birth does not automatically imply the inheritance of a disease state and could equally well be a shared physiological response to a common exposure. To discern the role of DNA methylation in the transmission of an epigenetic memory, it is necessary to examine the persistence of these changes into postnatal life, after the removal of the toxicant or disease state. In this regard, studies of paternal exposures offer a unique opportunity to determine the role of DNA methylation in transmission of environmentally induced phenotypes. Numerous reports have described the paternal transmission of adverse phenotypes in response to a range of preconception environmental and lifestyle exposures [38]. For example, experiments in rodents have associated alterations in both offspring behavior and phenotype with sperm-inherited epigenetic changes induced by diet, stress, and exposure to drugs of abuse [39–42]. Similarly, studies in humans have identified alterations in sperm-inherited patterns of DNA methylation induced by obesity as well as intense physical training [43,44]. However, evidence to both support and refute the involvement of sperm-inherited changes in DNA methylation in the transmission of the observed phenotypes has been reported [40,45–51], and the ability of DNA methylation to truly represent a form of transgenerational memory remains, as yet, unresolved. As an example, using a rat model, a recent study examining the transgenerational effect of Atrazine after a single exposure in the F0 mothers revealed hundreds of differentially methylated regions in sperm across three generations [52]. However, although the effect of offspring phenotype was consistent, there was negligible overlap in any of the identified differentially methylated regions across generations [52]. This illustrates clearly that the role of DNA methylation in driving the

transgenerational inheritance of environmentally induced phenotypes remains unresolved.

5. Context is King: statistical significance verses biological significance

As described previously, not all instances of environmentally induced transcriptional change can be explained by alterations in DNA methylation, nor are all statistically significant changes biologically meaningful. To this point, only a small proportion (15–20%) of CpGs responds to the environment and the majority of these map to gene enhancers [53]. Recent work by Curtis et al. [54] identified a number of differentially methylated enhancer regions in a patient cohort exposed to polybrominated biphenyl. Importantly, both the magnitude and direction of change identified in these enhancers were similar to those observed in studies examining changes in DNA methylation induced by estrogen, particularly in enhancer elements regulating the expression of the cytochrome P450 family genes [55]. Given the convergence of the aryl hydrocarbon receptor and estrogen pathways on these shared enhancer elements [56], the identification of these loci in both studies supports the assertion that the observed changes are part of a shared response to the induction of the coregulated genes. A similar study identified hypomethylation in a core set of enhancers containing predicted aryl hydrocarbon receptor binding motifs and used luciferase constructs to demonstrate the responsiveness of these regions to cigarette smoke [13]. These studies highlight the power giving functional context to observed changes in DNA methylation have in deciphering disease phenotype and in trying to understand the lasting impact of environmental exposures. The ENCODE database now contains functional DNA elements (enhancers and promoters) identified in a wide range of human and mouse tissues from across different developmental/life stages. To move forward, researchers in the field of developmental programming need to leverage this resource and give functional context to the reported changes. Only by applying this context will we be able to distinguish epigenetic changes that are a functional consequence of environmentally induced alterations in gene expression from those that appear as additional symptoms of exposure. Although most environmentally induced alterations in DNA methylation are not likely to drive altered patterns of gene expression, studies indicate that they do offer a molecular fossil record of past exposures [14]. Therefore, there remains some interest in using these signatures as biomarkers. However, most studies of environmentally induced changes in DNA methylation report low-magnitude changes at distinct cohorts of genes. Future studies are needed to determine how consistently these changes can be measured and if any genetic loci can be reproducibly associated with toxicant exposure in a clinical setting.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** of outstanding interest

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