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SciVerse ScienceDirect
Journal of Genetics and Genomics 40 (2013) 331−338
JGG

REVIEW

DNA Methylation, Behavior and Early Life Adversity

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Received 18 April 2013; revised 16 June 2013; accepted 19 June 2013
Available online 25 June 2013

ABSTRACT

The impact of early physical and social environments on life-long phenotypes is well known. Moreover, we have documented evidence for gene−environment interactions where identical gene variants are associated with different phenotypes that are dependent on early life adversity. What are the mechanisms that embed these early life experiences in the genome? DNA methylation is an enzymatically-catalyzed modification of DNA that serves as a mechanism by which similar sequences acquire cell type identity during cellular differentiation and embryogenesis in the same individual. The hypothesis that will be discussed here proposes that the same mechanism confers environmental-exposure specific identity upon DNA providing a mechanism for embedding environmental experiences in the genome, thus affecting long-term phenotypes. Particularly important is the environment early in life including both the prenatal and postnatal social environments.

KEYWORDS: DNA methylation; Epigenetics; Demethylase; Stress; Glucocorticoids; Maternal care; Early life adversity; Socioeconomic status

INTRODUCTION

DNA methylation is a covalent modification of DNA that is introduced into DNA by an enzymatic reaction catalyzed by DNA methyltransferases (Adams et al., 1979, 1981). During gestation, tissue specific DNA methylation patterns are formed (Razin and Szyf, 1984). It was proposed more than 3 decades ago that differential DNA methylation states between tissues play a critical role in defining tissue specific gene expression patterns (Razin and Riggs, 1980). In the early eighties, a series of studies established the existence of tissue specific DNA methylation patterns and the involvement of changes in DNA methylation patterns in tissue and cell differentiation. These results have been confirmed by recent whole genome sequencing methods (Lister et al., 2009). Although the exact role of DNA methylation in regulating gene function has been under intense discussion in the last two decades, several general principles have been established. DNA methylation in 5′ regulatory region silences promoter activity either through direct interference with binding of transcription factors (Comb and Goodman, 1990) or through recruitment of methylated DNA binding protein that attracts chromatin silencing complexes to methylated regions (Nan et al., 1998). It is clear that DNA methylation occurs in bodies of genes where it was proposed to simulate expression through unknown mechanisms. It was proposed also that DNA methylation is involved in mRNA splicing. DNA methylation is tightly linked with other chromatin modification mechanisms and as mentioned above DNA methylation could lead to the formation of silencing chromatin (Eden et al., 1998), and chromatin-modifying enzymes could recruit DNA methylating enzymes (Fuks et al., 2003).

DNA METHYLATION IN THE FACE OF DYNAMIC ENVIRONMENTS

The fact that DNA methylation plays an important role in specifying cell type specific programs (Razin and Riggs, 1980) implies that DNA methylation will be tightly conserved during the life span of a tissue and it was therefore believed that DNA methylation patterns are highly fixed and resistant to changes

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http://dx.doi.org/10.1016/j.jgg.2013.06.004

in somatic tissues. The maintenance DNA methyltransferase 1 (DNMT1) maintains the pattern of methylation in dividing cells by faithfully copying methylation sites from the template strand to the nascent DNA strand during cell division (Gruenbaum et al., 1982). The postulated absence of *de novo* methyltransferases (DNMT), enzymes that add new methylation sites, in somatic cells and demethylases were believed to ensure that the DNA methylation pattern was maintained and preserved in a tissue specific manner across different individuals. These basic concepts almost precluded the possibility that DNA methylation might vary in response to external exposures such as environmental experiences. However, data emerged in the last decade suggest that DNA methylation could be involved in conferring exposure-specificity on DNA and that these DNA methylation differences are associated with stable phenotypes (Szyf, 2012). This idea is of particular relevance for mental health and behavioral biology where gene−environment interactions have been documented for decades (Caspi et al., 2003) and the impact of the external social environment on behavior and brain development were extensively documented.

WORKING HYPOTHESIS: DNA METHYLATION IS A POTENTIAL GENOMIC MECHANISM FOR ADAPTING LONG-LASTING GENOME PROGRAMS TO THE SOCIAL ENVIRONMENT

DNA methylation is now a well-established mechanism for embedding cell-type identity in DNA in response to intrinsic developmental signals. However, a significant component of human and animal development is known to be responding to external environmental signals. This is particularly relevant to mental functions and behavior, immunity and inflammation as well as metabolic health. We therefore propose that DNA methylation participates in sculpting genome function in response to signals from the environment. The early life period is particularly important, and social and physical environmental cues at this time point will trigger programming of the genome in anticipation of life-long environmental exposures. It is important to note that early life adverse environments deliver signals that affect immunity, cardiovascular and mental responses and it is therefore hypothesized that early life adversity alters DNA methylation in multiple physiological systems that include both the brain and peripheral systems. We also proposed that these might be integrated responses as adverse environments usually affect these systems concurrently.

First evidence for environmental programming of life-long phenotypes early in life that is mediated by changes in DNA methylation came from Randy Jirtle's lab. The group demonstrated that maternal diet during gestation affects the agouti color phenotype in yellow agouti (A(vy)) mice, and this effect was mediated through methylation of a transposable element in the A(vy) mouse (Waterland and Jirtle, 2003) while exposure of the mother to bisphenol B results in demethylation of the same element. This was a first demonstration that maternal nutrition can have a long-term effect on the phenotype of the offspring and that an external environmental signal could affect the emergence of a stable distinctive DNA methylation pattern. Similarly, bisphenol A shifted the coat color of the offspring toward yellow by decreasing methylation in a retrotransposon upstream of the *agouti* gene (Dolinoy et al., 2007). However, it wasn't clear whether this effect reflected a programmed rearrangement of the DNA methylation in response to an environmental signal or just a stochastic consequence of chemicals inhibiting the DNA methylation enzymes in development during a critical time when DNA methylating enzymes are laying down the DNA methylation pattern. The main question is whether there is a mechanism that programs DNA methylation in response to environmental exposures during prenatal and early postnatal periods. The main evidence for a programmed response to environment came from studies showing that DNA methylation changes could occur in response to experiences including social experiences that do not involve exposure to particular extraneous chemicals but involve a programmed interpretation of these experiences. The new field of behavioral epigenetics has provided the first evidence for a programmed response to experience that highlighted the importance of the early life.

THE IMPACT OF EARLY LIFE ADVERSITY ON THE PHENOTYPE

The early life social environment is known from epidemiological studies to be associated with health outcomes later in life and these include physical and mental health such as cardiovascular, metabolic and mental health challenges (Power et al., 1997; Hertzman et al., 2001; Power et al., 2006). Early life adversity is associated with depression and psychiatric disorders later in life (Maughan and McCarthy, 1997; Hazel et al., 2008; McLaughlin et al., 2010; Stein et al., 2010; Uchida et al., 2010; Angst et al., 2011). The earliest evidence for gene−environment interactions came from studies showing individuals with a polymorphism in the serotonin transporter gene (*5HTT*) (short allele) will exhibit more depressive symptoms, diagnosable depression, and suicidality when experiencing stressful life events (Caspi et al., 2003). These data suggest that there must be a mechanism by which the environment affects gene function; however, gene−environment interaction remains an epidemiological concept with no concrete biochemical mechanism.

THE EMERGENCE OF BEHAVIORAL EPIGENETICS: THE EFFECT OF MATERNAL CARE

The mother is the principal early social environment of a mammal and maternal care plays a critical role in the future well being of her offspring. Nonhuman primate models of maternal deprivation have recapitulated the effects seen in humans that experienced social adversity early in life (Ruppenthal et al., 1976; Suomi et al., 1976). The first set of evidence that epigenetic processes mediate the programming of behavior by early life experiences came from studies in

rodents. We used a model of maternal care in rats developed by Meaney's lab to examine whether natural variations in maternal care just after birth cause DNA methylation differences that might be behind the observed phenotypic differences between the adult offspring that experienced either High Licking and Grooming (H) or Low Licking and Grooming (L) maternal care. Our original approach focused on stress response and on a candidate gene regulating the stress response, the glucocorticoid receptor (GR exon 1_7 promoter) in the hippocampus, a site of feedback inhibition of glucocorticoid release in response to stress. The hypothalamic-pituitary-adrenal (HPA) axis is particularly affected in the offspring of L mothers with heightened glucocorticoids in response to stress. This effect is epigenetic since cross fostering of the offspring results in an adult phenotype of the offspring, which is dependent on the fostering and not on the biological mother (Francis et al., 1999). Weaver et al. (2004) showed that variations in maternal care result in differences in epigenetic programming by histone acetylation and DNA methylation of the exon 1_7 promoter of the GR in the hippocampus resulting in differences in expression of GR which in turn results in blunted negative feedback inhibition by glucocorticoids and a heightened stress response. The difference emerging early after the maternal behavior of licking and grooming is initiated and remains into adulthood (Weaver et al., 2004).

REVERSIBILITY OF DNA METHYLATION IN THE BRAIN

The fact that these changes in DNA methylation occur in a postmitotic tissue after birth and in response to an external social signal suggests that the DNA methylation is reversible and can therefore function as a true physiological signal in response to the environment. We have previously shown that an isoform of the methylated DNA binding protein 2 (MBD2) bears an active demethylation activity (Bhattacharya et al., 1999; Ramchandani et al., 1999). However, this finding was hotly contested (Ng et al., 1999; Hendrich et al., 2001). Additional mechanisms for cell division-independent demethylation (Razin et al., 1986; Jost, 1993) that involve either deamination (Kangaspeska et al., 2008) or hydroxylation followed by base excision repair were more recently proposed (Kriaucionis and Heintz, 2009). More recently, it was proposed that the DNA methylating enzymes DNMT1, DNMT3A and DNMT3b could also catalyze the reverse reaction demethylation (Chen et al., 2013). The nature of the enzymatic activity in the brain that removes demethylation needs to be determined.

We used the maternal care model to test whether DNA methylation programmed by maternal care is reversible in the adult brain. We used the histone deacetylase inhibitor (HDACi) trichostatin A (TSA) to increase histone acetylation and induce active demethylation in the hippocampus. We have previously shown that histone acetylation could promote replication-independent demethylation in cultured cells (Cervoni and Szyf, 2001). TSA caused demethylation and a phenotypic change in the behavior of the offspring of the Low maternal care mothers that became indistinguishable from the behavior of the offspring of High maternal care mothers (Weaver et al., 2004). Conversely, injecting methionine into the brain increased methylation in the brain and reversed the behavior of High maternal care mothers that became indistinguishable from the behavior of offspring of Low maternal care mothers (Weaver et al., 2005). These experiments demonstrated that DNA methylation changes in the brain in a stable way in response to early life social signals, but in spite of the persistence of this effect it could be reversed pharmacologically in the adult brain. This illustrates the special promise of DNA methylation modulators in future treatment of behavioral and psychiatric disorders. Moreover, these experiments highlighted the impact of social experience on DNA methylation, raising the possibility that behavioral therapy could be used in the future to treat and reverse epigenetic marks triggered by early life social adversity. More recently, our results with rats were extended to mice. Demethylation of GR promoter was correlated with increased maternal care in juvenile Balb/c mice (Liberman et al., 2012).

These results in rodents were translated to humans as well. We used postmortem hippocampal samples from humans that were abused in childhood and were victims of suicide, victims of suicide who were not abused as children and control subjects who died from other causes. We demonstrated that the promoters of ribosomal RNA (*rRNA*) genes as well as the promoter of the GR exon 1f (homologue of the 1_7 promoter in humans) were hypermethylated and less expressed in brains of victims of child abuse (McGowan et al., 2008, 2009).

Other candidate genes were shown to be differentially methylated in response to social adversity in different rodent models. Exposure of infant rats to abusive stressed caretakers produced long-lasting alterations in the state of methylation of *brain derived nerve growth factor* (*bdnf*) gene promoter in the adult prefrontal cortex (Roth et al., 2009). Similarly, early-life stress (ELS) in mice caused sustained DNA hypomethylation of an important regulatory region of the *arginine vasopressin* (*AVP*) gene (Murgatroyd et al., 2009).

BROAD GENOME-WIDE EFFECTS OF EARLY LIFE ADVERSITY

The early studies testing a link between social experience and DNA methylation focused on candidate genes that were centrally positioned in physiological pathways responsive to social adversity. Epidemiological data suggest that the effects of early life adversity are widespread. Since it is clear that genes don't work on their own, we tested the hypothesis that the impact of early life stress would not be limited to few genes but that it would be widespread and would affect several functional gene networks. We tested this hypothesis and showed that differences in maternal care resulted in a highly organized clustered and broad change in DNA methylation, histone acetylation and gene expression (McGowan et al., 2011). Particularly notable were changes in DNA methylation and gene expression in the *protocadherin* family of

genes that covered the entire cluster (McGowan et al., 2011). These genes were previously shown to be involved in synaptogenesis in the brain (Garrett and Weiner, 2009) and were discovered to be epigenetically regulated by maternal care by our unbiased screen. Remarkably, we noted high evolutionary conservation of these clustered and broad changes in DNA methylation in response to early life adversity in human brains. The *protocadherin* gene cluster was differentially methylated with relation to early life experience in humans as well as rats (Suderman et al., 2012).

CHANGES IN DNA METHYLATION IN RESPONSE TO EARLY LIFE ADVERSITY ARE SYSTEM WIDE

Having proven that alterations in DNA methylation in the brain in response to child adversity are not limited to few candidate genes, we tested whether the changes were present in the periphery as well. This question has important implications for behavioral epigenetics. First, although we have been studying the brain and the body independently in both basic sciences and practice of medicine, it is clear that they are integrated. Moreover, social adversity doesn't signal just social adversity but it is tightly linked to nutritional and biological adversity. Therefore, it stands to reason that the response to adversity will be coordinated across the body. Second, there is an important practical implication. It is impossible to study DNA methylation in brain of living subjects, particularly using DNA methylation to predict behavioral pathology and to follow up interventions. Longitudinal studies in living subjects are critical. Progress of behavioral epigenetics is dependent on identifying informative DNA methylation markers in white blood cells or other noninvasive tissue source. An additional limitation of human studies is determining whether the DNA methylation changes are immediate results of social adversity early in life or a consequence of the phenotypes associated with early life adversity. The only way to address this question is to conduct randomized studies with early life adversity, but this is impossible in humans.

To address these questions, we resorted to a nonhuman primate model that exhibited the complex behaviors seen in humans that could not be recapitulated in rodent models. Suomi et al. (1976) have developed a model of early life adversity that involves randomized maternal deprivation of rhesus macaques monkeys early after birth. These monkeys exhibit many of the phenotypes seen in humans exposed to early life adversity (Ruppenthal et al., 1976; Suomi et al., 1976; Miller et al., 2008; Corcoran et al., 2012). We asked three critical questions: First, does randomized early life adversity trigger DNA methylation change? Second, are the DNA methylation changes that result from social adversity limited to the brain or could they be found in other tissues? Third, is there a correspondence between DNA methylation changes in specific cell types in blood such as T cells and specific regions in the brain such as the prefrontal cortex?

Our results showed that there is a signature of maternal care in the DNA methylation pattern in T cells as well as the brain. These changes are highly clustered and organized and involve critical pathways including genes, which play a particular role in the immune system as well as the HPA axis. Since these monkeys were randomized for early life adversity at birth, the study demonstrates a causal relationship between the adverse experience early in life and DNA methylation changes in adulthood. The changes in the brain and T cells may target different regions as expected if these cell types play different physiological roles in the response to child adversity. However, in addition to these tissue specific differentially methylated regions, we identified regions that were similarly differentially methylated in the brain and T cells (Provencal et al., 2012). These studies demonstrate that it is feasible to study behaviorally related DNA methylation signatures in peripheral cells, particularly T cells. The immune system functionally interacts with the brain particularly the HPA axis and there is evidence that cytokines play some roles in brain function (Raber et al., 1998; Zalcman et al., 1998; Yirmiya and Goshen, 2011). So it stands to reason that there is a bilateral discourse between T cells and the central nervous system in response to adversity including social adversity.

A related question is whether these changes in DNA methylation were triggered by the early life adversity or by downstream environmental and experiential differences between the groups. Unpublished data in our lab suggest that these changes emerge very early after randomized adversity is initiated.

PATHWAYS LINKING SOCIAL ADVERSITY AND DNA METHYLATION ALTERATIONS

The functional and structural organization of the response to early life adversity suggests that similar to the developmental pathways that organize DNA methylation changes during development, there are signaling pathways that serve as a conduit between the social experience and the epigenome. In addition, there must be mechanisms that integrate the response across tissues as is implicated by the results in the rhesus differential rearing model. We have previously proposed that a cascade of signaling pathway links maternal care and changes in DNA methylation in the GR exon 1_7 promoter as well as other broad regions described in our studies. It was suggested that serotonin (5-HT) acting through one of its receptors and released in response to maternal care induces the expression of cAMP and protein kinase A, which in turn activates the transcription factor NGFIA (nerve growth factor induced clone A). This transcription factor delivers DNA demethylating and histone acetylation activities to its targets resulting in targeted histone acetylation and DNA demethylation (Meaney and Szyf, 2005; Weaver et al., 2007).

Another signaling pathway that has been proposed to result in demethylation and activation of *AVP* in neurons of the hypothalamic paraventricular nucleus (Murgatroyd et al., 2009) involved the methylated DNA binding protein 2 (MeCP2), probably through phosphorylation of MeCP2 by calmodulin kinase II (CamKII) in response to neuronal activation. Phosphorylation of MeCP2 was previously suggested to promote

demethylation and activation of gene expression in neurons (Zhou et al., 2006). Perhaps the most important signaling pathway that regulates DNA methylation in response to early life stress involves interaction of the glucocorticoid hormone released by stress and its receptor with target genes.

SYSTEM WIDE EPIGENETIC INTEGRATORS OF RESPONSES TO EARLY LIFE STRESS

One of the most critical roadblocks for behavioral epigenetics is the question of whether it is possible to derive data informative for behavior from peripheral cell types particularly white blood cells. There is increasing evidence that DNA methylation alterations in blood could be associated with early life experiences. For example, the glucocorticoid receptor (*NR3C1*) promoter is more methylated in lymphocytes in newborns exposed prenatally to maternal depression than control newborns (Oberlander et al., 2008). *Pituitary adenylate cyclase-activating polypeptide* (*PACAP*) was found to be differentially methylated in peripheral blood cells in humans with post-traumatic stress syndrome (Ressler et al., 2011). Deficiencies in early life nurturing were associated with increased methylation of *NR3C1* promoter in leukocytes (Tyrka et al., 2012) and maternal anxiety during gestation was associated with methylation of the NGFIA (nerve growth factor induced clone A) binding site in *NR3C1* in cord blood (Hompes et al., 2013). Early life low social economic positioning was shown to associate with a distinct DNA methylation signature in white blood cells (Borghol et al. 2012). The data to date support therefore the hypothesis that the DNA methylation profiles of early life adversity could be detected in white blood cells including isolated cell types such as T cells. It also appears that although many of the responses seen in T cells and white blood cells relate to immune function, certain differentially methylated genes in blood have known functions in the brain. The changes in immune specific genes are consistent with the hypothesis that the response to child adversity is system wide and that different physiological systems are adapting to the environmental signals in early life as discussed above. The immune system is known to be responsive to stress and early life conditions (Miller et al., 2009). However, it is more difficult to postulate that DNA methylation differences in T cells would provide functional information on changes in the brain. Although it is impossible to test the state of DNA methylation in brain and compare it to T cells in the same living subjects, it might be possible to make predictions on the possible functional pathways that might be affected in the brain if the gene is methylated. For example, we have recently shown in a small number of subjects that the 5-HT transporter *SLC6A4* promoter is more methylated in subjects that were aggressive during their childhood in T cells and monocytes. Using positron emission tomography (PET) measures of brain 5-HT synthesis, we found lower *in vivo* 5-HT synthesis in the orbitofrontal cortex (OBFC) (Wang et al., 2012). These data raise two important questions. First, do similar changes in DNA methylation occur in both brain and peripheral cells? Second, what are the signals that coordinate DNA methylation changes across the body targeting the same genes in multiple tissues? Addressing these questions is critical for understanding the mechanisms that link life-long phenotypes across multiple physiological systems with early life adversities.

A recent study examined a gene−environment interaction between SNPs in the *FK506 binding protein 5* (*FKBP5*) gene encoding a protein that regulates glucocorticoid responses and child adversity; the combination of a polymorphic allele and exposure to child adversity increases the risk to PTSD (post-traumatic stress disorder). The authors showed that a combination of genetic variation and exposure to early life stress was associated with demethylation of a glucocorticoid response element in the stress response regulator *FKBP5* in white blood cells (Klengel et al., 2013). This study confirmed that differential DNA methylation associated with behavioral pathologies could be found in white blood cells. The authors also showed that glucocorticoid exposure of human progenitor hippocampal neuronal cells results in the same demethylation. Interestingly, the demethylation occurs only prior to differentiation of the cells, consistent with the idea that there is a critical window

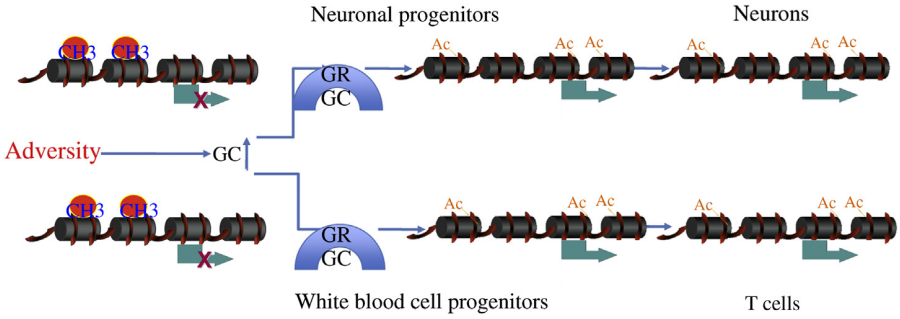

Fig. 1. Glucocorticoids integrate DNA methylation responses to early life adversity, a model.
Early life adversity results in excessive release of glucocorticoids (GCs) which circulate. GCs interact with the glucocorticoid receptors (GRs) in neuronal and white blood cell progenitors. The bound receptor interacts with common target methylated genes (CH3) which are silenced (indicated by X on horizontal arrow), recruits demethylase activity and demethylates similar genes in neuronal and white blood cell progenitors. The gene becomes demethylated and active in progenitor cells. The progenitors develop into mature neurons and T cells bearing similar DNA demethylation and transcription activation in common targeted genes. Ac, histone acetylation.

when glucocorticoids could exert this effect (Klengel et al., 2013). Based on these data, a mechanism for coordinated changes in DNA methylation in the brain and periphery in response to early life adversity could be proposed (Szyf, 2013). Early life adversity results in heightened release of glucocorticoids. Since receptors for glucocorticoids are present in most tissues particularly in immune cells and brain cells, they could simultaneously target the same gene in multiple tissues and GR was shown to target DNA demethylation (Thomassin et al., 2001). Hormones such as glucocorticoids could therefore integrate DNA methylation responses to experiences early in life across many tissues (Fig. 1). Further data are needed to support this hypothesis; however, if true, this could revolutionize our understanding of behavioral epigenetics. We have recently shown that antenatal exposure of guinea pigs to synthetic glucocorticoids during gestation results in DNA methylation changes in the brain as well as peripheral tissues that cross generations (Crudo et al., 2012, 2013). These data support the hypothesis that glucocorticoids act as coordinators of DNA methylation responses across tissues.

SUMMARY

Data reviewed here suggest that DNA methylation is a candidate to serve as mechanism that responds to external experiences and alters gene function and phenotypes in a stable manner. DNA methylation serves therefore as a mechanism conferring specific functions to identical DNA sequences in response to different life experiences. These responses are system wide and are not limited to the brain since social adversity has important physical implications. A possible mechanism that integrates DNA methylation responses across tissues are circulating hormones such as glucocorticoids that are released in response to adversity and could act on similar targets in multiple tissues. The fact that we could detect DNA methylation differences in peripheral tissues has important practical implications.

ACKNOWLEDGEMENTS

Work in M.S. lab is supported by a grant from the Canadian Institute of Health Research to M.S. M.S. is a fellow of the Canadian Institute for advanced research. M.S. is supported by the Sackler program in epigenetics and psychobiology at McGill University, Canada.

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