



Epigenomics in environmental health

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This review considers the emerging relationships between environmental factors and epigenetic alterations and the application of genome-wide assessments to better define these relationships. First we will briefly cover epigenetic programming in development, one-carbon metabolism, and exposures that may disrupt normal developmental programming of epigenetic states. In addition, because a large portion of epigenetic research has focused on cancer, we discuss exposures associated with carcinogenesis including asbestos, alcohol, radiation, arsenic, and air pollution. Research on other exposures that may affect epigenetic states such as endocrine disruptors is also described, and we also review the evidence for epigenetic alterations associated with aging that may reflect cumulative effects of exposures. From this evidence, we posit potential mechanisms by which exposures modify epigenetic states, noting that understanding the true effect of environmental exposures on the human epigenome will require additional research with appropriate epidemiologic studies and application of novel technologies. With a more comprehensive understanding of the affects of exposures on the epigenome, including consideration of genetic background, the prediction of the toxic potential of new compounds may be more readily achieved, and may lead to the development of more personalized disease prevention and treatment strategies.

Keywords: exposures, asbestos, aging, arsenic, endocrine disruptors, metals, DNA methylation, microRNA

INTRODUCTION

Epigenetics can be broadly defined as mitotically and meiotically heritable changes in gene expression and gene expression potential that cannot be explained by changes in DNA sequence. Examples of epigenetic states that can be altered include methylation of cytosine residues on DNA, post-translational modification of histone tails, and microRNA (miRNA) expression. DNA methylation is the most widely studied epigenetic mark and refers to the catalytic addition of a methyl group to the fifth carbon position of a cytosine residue that is followed on the same strand by guanine, also known as a CpG dinucleotide. Though underrepresented in the genome, CpG dinucleotides occur in concentrations known as CpG-islands, and CpG-islands can be found in the promoter region of approximately half of all human genes. In pathologically normal cells promoter CpG-island regions are typically unmethylated. Aberrant methylation of a CpG-island can result in gene silencing, and this is a well-recognized mechanism by which epigenetic alterations can contribute to a disease phenotype.

Epigenetic regulatory mechanisms are stable mechanisms for the control of gene expression, though they also display some level of plasticity, likely in response to various cues from the environment. Thus, it is increasingly important to characterize the role for environmental exposures in epigenetic alterations. Much of the existing evidence implicating a role of environmental factors on the epigenome comes from studies of disease outcomes such as cancer and adverse reproductive/developmental events, although there is a growing literature in the role of the psychosocial environment's impact on the epigenome as well. Initially, epigenetic alterations were identified in various human tumors

and consequently, environmental exposures to toxicants known to have an etiologic role in cancer such as air pollutants, and metals have been studied and implicated in the modification of epigenetic marks in pathologic tissues.

To better understand the importance and function of such alterations in disease states, we might begin to consider how environmental factors may influence the epigenome in pathologically normal tissues, perhaps representing alterations necessary to initiate or predispose to disease phenotypes. To date, the majority of epigenetic research in human studies has focused on DNA methylation, though emerging work is beginning to address the affect of environmental exposures on histone modifications and miRNA expression. This review highlights the current evidence that epigenetic alterations are associated with environmental exposures, focusing on human studies, and considers through this research the potential mechanisms behind environmentally related epigenetic alterations.

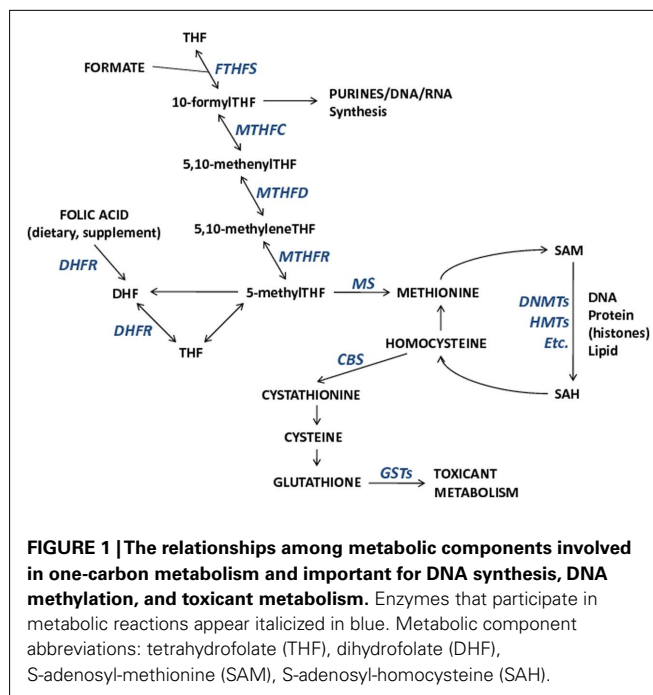
DEVELOPMENT AND EPIGENETIC PROGRAMMING

The establishment of somatic cell epigenetic patterns, in mammals, occurs early in fetal development. The re-establishment of appropriate epigenetic patterns is subsequent to genome-wide demethylation following fertilization, required for allowing totipotency and pluripotency dynamics (Morgan et al., 2005). Thus, embryonic and fetal development represent a critical period during which nutrient availability as well as environmental stressors, including toxicant exposures, have great potential to affect the epigenetic reprogramming and patterning phenomena. Thus, these effects can not only have implications for proper development,

but perhaps even life-long conditioning and health (Li, 2002; Gluckman et al., 2008). For instance, it is well-known that folate deficiency is associated with neural tube defects (NTD; Daly et al., 1995), animal studies have indicated the necessity of sufficient methyl group availability for proper neural tube development (Dunlevy et al., 2006), and hypomethylation of long interspersed nucleotide elements (LINE) and genomic DNA has been associated with increased risk of NTDs in humans (Wang et al., 2010). Beyond birth, though, epigenetic mechanisms may contribute to the developmental origins of health and disease hypothesis (Waterland and Michels, 2007), which links nutritional and environmental stimuli at critical developmental periods (pre- and post-natal) to susceptibility for various health outcomes throughout life, including metabolic and chronic diseases (Gluckman and Hanson, 2004).

The importance of folate, and other nutrients involved in one-carbon metabolism on the establishment and maintenance of DNA methylation and potentially other epigenetic mechanisms is one mode through which toxicants may have epigenome-wide effects. One-carbon metabolism is the network of biochemical reactions essential to both DNA synthesis and all cellular biomolecule (nucleic acids, proteins, lipids) methylation reactions that involve the transfer of one-carbon groups. Folate (vitamin B₉), a central nutrient in this pathway, donates its methyl group for homocysteine remethylation to methionine. Subsequently, methionine is the methyl donor for all cellular methylation reactions, most notable for this discussion being DNA and histone methylation via S-adenosyl-methionine (SAM), and other B vitamins (B₂, B₆, B₁₂) that act as enzymatic cofactors in the network. Collectively, B vitamins, homocysteine, and methionine are important contributors to the maintenance of DNA integrity and DNA methylation. The one-carbon pathway links nutrient availability with not only DNA methylation, but also with toxicant metabolism through glutathione and the glutathione transferase (GST) enzyme family (Figure 1). Therefore, interactions between one-carbon pathway participants may modify associations between exposures, pathway participants, and methylation alterations. In fact, a study of reduced GST enzyme activity in a mouse model of Alzheimer's disease and neuronal health has shown that SAM can mediate the activity of GST enzymes (Tchantchou et al., 2008). This suggests that reactions dependent upon SAM are necessary for GST activity and that SAM may be a critical mediator of neuronal health (Tchantchou et al., 2008). Furthermore, it could be predicted that GST enzyme family genotypes (among other genotypes) will be shown to modify associations between certain exposures and DNA methylation alterations. Hence integrative studies that incorporate genetics (genotype and/or alterations) with measures of epigenetic alterations will allow a more comprehensive understanding of the relation among exposures, epigenetic alterations, and genetic states.

The key role of one-carbon metabolism also brings to light the potential risks of high-dose nutrient supplementation practices. Folate supplementation is associated with a reduced risk of many forms of cancer, though once disease is present it is possible that a relative increase in one-carbon nutrient levels may speed disease progression or invasiveness dependent upon the epigenetic character of the disease state. Some evidence for this



potential comes from our recent study of breast tumor methylation where increased folate intake (controlling for potential confounders) was associated with a methylation profile that was independently associated with increased tumor size (Christensen et al., 2010). Excessive folate supplementation may have potential adverse effects including masking of B₁₂ deficiency, disruption of zinc function, or interfering with one-carbon homeostasis and additional study is required to elucidate these potential effects (Hathcock, 1997; Sauer et al., 2009). In addition, more research is needed to ascertain best practices for assessing folate/methyl availability, perhaps comparing food-frequency-questionnaire data to homocysteine and/or SAM/SAH levels in a prospective manner.

TOXICANTS AND EPIGENETIC ALTERATIONS

ALCOHOL

Alcohol is known to interfere with folate absorption in the intestine and hepatic release of folate, and hence, supply to tissues (Hillman and Steinberg, 1982) and thus it too can be considered a toxicant with the potential for epigenomic alteration. More specifically, through interference with several steps of methionine metabolism alcohol can lead to activation of betaine homocysteine methyltransferase thereby activating a compensatory pathway for maintenance of SAM levels (Kharbanda, 2009). Continuous exposure though, can overload the compensatory pathway and result in liver injury. In addition to liver injury, an etiologic role for alcohol in several cancers is well established (International Agency for Research on Cancer, 1988). Alcohol consumption increases colon cancer risk (Giovannucci et al., 1995), though the association may be modified by dietary folate, and may be specific to LINE-1 hypomethylated tumors (Schernhammer et al., 2010). More specifically, subjects with high folate intake were less likely to develop LINE-1 hypomethylated colon cancers but subjects with high alcohol consumption had a significantly increased risk of

developing LINE-1 hypomethylated colon cancers with no association for more highly methylated LINE-1 tumors (Schernhammer et al., 2010). In addition, in head and neck squamous cell carcinoma, where alcohol has a well-recognized etiologic role, alcohol consumption has been significantly associated with reduced LINE-1 methylation (Smith et al., 2007). Profiles of DNA methylation in HNSCC tumors are significantly and independently associated with alcohol intake (Marsit et al., 2009), suggesting that alcohol use may drive or select for the epigenetic alteration of specific gene regions. In multiple meta-analyses of prospective and case-control studies of breast cancer, a 10% excess risk for each alcoholic drink per day has been reported (Hamajima et al., 2002; Key et al., 2006). A study of breast tumor DNA methylation profiles demonstrated a significant, independent association (controlling for age, dietary folate, and other variables) between alcohol intake and tumor DNA methylation profile (based on 1413 CpG loci; Christensen et al., 2010). In addition to DNA methylation, ethanol exposure has been shown to selectively acetylate histone H3 at lysine 9 (H3K9) in primary culture of rat hepatocytes (Park et al., 2003) and in rats *in vivo* (Kim and Shukla, 2006), though studies in humans are necessary. Collectively, these studies suggest that a major carcinogenic mechanism of action of alcohol is interference with epigenetic regulation, in part through disruption of one-carbon metabolism.

ASBESTOS

Despite the known risks of exposure, asbestos continues to be mined and exported to developing nations where occupational exposures can be high. With approximately 80% of cases reporting a known exposure to asbestos (Robinson and Lake, 2005), asbestos exposure is the main risk factor for malignant pleural mesothelioma. Importantly, in contrast to tobacco smoke and radiation, asbestos is known to be a weak mutagen (Sugarbaker et al., 2008), and there have been several reports of tumor suppressor gene methylation in mesothelioma (Ohta et al., 1999; Hirao et al., 2002; Lee et al., 2004; He et al., 2005). In patients with mesothelioma, Tsou et al. (2007) described a significant association between self-reported asbestos exposure and methylation at the *MT1A*, and *MT2A* gene loci among 28 genes examined. A significant association has been reported between an increasing number of methylated cell cycle control genes and a quantitative asbestos burden measure (Christensen et al., 2008). This was followed by examination using an array-based approach, which demonstrated that quantitative measure of asbestos exposure was associated with over 100 discrete CpG loci, and that in almost all cases (94%) there was increased methylation associated with increased exposure (Christensen et al., 2009a). Further, overall methylation profiles for mesotheliomas were significantly associated with asbestos exposure burden (Christensen et al., 2009a).

ARSENIC

Arsenic exposure has been associated with DNA methylation alterations in non-pathologic as well as tumor tissues and there is some data suggesting that changes in miRNA expression and histone tail modifications are also associated with exposure to arsenic. Although the mode (or modes) by which arsenic contributes to carcinogenesis is not completely clear, there is some speculation that arsenite-generated free radicals and arsenite-generated

reactive oxygen species lead to genotoxic damage (Rossman, 2003). Despite continued speculation on the mechanisms by which arsenic can contribute to carcinogenesis, *in vitro* exposures to inorganic arsenic species have been shown to result in altered DNA methylation (Zhao et al., 1997; Zhong and Mass, 2001).

As more studies are conducted a complex picture of dose-dependent DNA methylation alterations with arsenic exposure is beginning to emerge. Chanda et al. (2006) have shown that arsenic exposure can result in *CDKN2A* hypermethylation in human blood DNA, but that a subgroup of cases had hypomethylation with high arsenic exposure. More recently, a group of some of the same authors has better quantified the relation showing that exposures to 250–500 µg/L of arsenic in drinking water results in global hypermethylation (³H methyl group uptake), but that >500 µg/L of arsenic results in global hypomethylation (Majumdar et al., 2010). In human bladder cancer, relatively low levels of inorganic arsenic exposure has been associated with methylation of *RASSF1A* and *PRSS3* (but not *CDKN2A*; Marsit et al., 2006b). A recent study of a large number of human bladder tumors that examined hundreds of genes demonstrated a relationship in promoter methylation profiles with arsenic exposures, suggesting that more highly methylated tumors came from individuals with larger drinking water arsenic exposures, and that these tumors were more aggressive (Wilhelm-Benartzi et al., 2010). In mouse models of methyl- or folate-deficient diets, arsenic exposure through water supply led to hypomethylation in hepatic-derived DNA (Okoji et al., 2002), as well as to increases in chromosomal aberrations in blood lymphocytes (McDorman et al., 2002). In another study, Pilsner et al. (2007) showed increased DNA methylation to be associated with urinary and plasma arsenic and plasma folate, and that the association between arsenic and methylation was modified by folate in that it was restricted to individuals with high plasma folate. This same group has shown that folic acid supplementation lowers blood arsenic (Gamble et al., 2007), maternal and cord blood pairs have highly correlated arsenic levels (Hall et al., 2007), and folate deficiency, hyperhomocysteinemia, and leukocyte hypomethylation are associated with arsenic-induced skin lesions (Pilsner et al., 2009). Thus, perhaps a more plausible mechanism for arsenic-related carcinogenicity is via the depletion of *S*-adenosyl-methionine (SAM, the universal methyl donor) due to the metabolism of inorganic arsenic to its methylated forms resulting in altered DNA methylation.

Beyond interfering with one-carbon metabolism and affecting DNA methylation, treatment of human lymphoblastoid cells with sodium arsenite led to global increases in miRNA expression (Marsit et al., 2006a). The genotoxic effects of arsenic may also be mediated by altered chromatin and Ramirez et al. (2008) have shown in human hepatocarcinoma cells that treatment with sodium arsenite resulted in global increases in histone acetylation. Thus, it is reasonable to posit that increased expression of miRNAs associated with arsenic exposure may be due to increased histone acetylation. As with other toxicants, additional studies are necessary to further elucidate the associations between arsenic and all major forms of epigenetic alteration with particular attention being given to dose and modification by one-carbon metabolism pathway participants.

OTHER METALS (NICKEL, LEAD, CADMIUM, CHROMIUM)

There is a growing body of evidence for epigenetic alterations due to exposure to other metals in humans; several studies have suggested that exposures to metals such as nickel, lead, cadmium, and others may alter epigenetic marks. *In vitro*, nickel exposure has been associated with various histone modification and decreased histone H4 acetylation (Broday et al., 2000; Ke et al., 2006). Exposure of a rat cell line to cadmium resulted in a concentration-dependent reduction in DNA methyltransferase activity (Takiguchi et al., 2003).

In a study among electric-furnace steel plant workers, experiencing metal-rich (lead, cadmium, chromium) particulate matter exposure has resulted in significant alterations of miRNA expression in peripheral blood leukocytes (Bollati et al., 2010). Patella lead levels, amongst participants in the Normative Aging Study were associated with reduced global DNA methylation (LINE-1 elements) though not Alu repeat regions (Wright et al., 2010), suggesting a role for lead exposure in epigenetic alterations. Interestingly, a sexually dimorphic global methylation (Luminometric methylation assay for CCGG) level decrease was associated with brain mercury levels in male polar bears (Pilsner et al., 2010).

The industrial applications of chromium include chrome plating and stainless steel welding and its exposure amongst those occupationally exposed as been related to lung cancers (Gibb et al., 2000). Beyond the recognized role for methylation induced silencing of *CDKN2A* in lung cancer that is related to tobacco smoking (Kim et al., 2001; Toyooka et al., 2003), Kondo et al. (2006) showed that chromate lung cancers were more likely to have methylated *CDKN2A* with increasing duration of occupational chromate exposure. An *in vitro* exposure of mouse hepatoma cells to chromium showed transcriptional repression of *Cyp1a1* by local cross-linking of Hdac and Dnmt1 and altered histone marks (Schnekenburger et al., 2007). However, additional study of this diminishing exposure is necessary to validate potential chromium-induced epigenetic alterations.

PARTICULATES AND AIR POLLUTION

Though few studies have addressed the potential for air pollution exposure to alter DNA methylation, environmental particulate exposure has been linked to increased risk of lung cancer and increased morbidity and mortality from cardiovascular and respiratory illnesses (Vineis and Husgafvel-Pursiainen, 2005). In rat lung cancer models of tumors induced by diesel exhaust, 59% were methylated at *Cdkn2a*, and 14% at *Esr1*, in those induced by carbon black 45% were methylated at *Cdkn2a* and 14% at *Esr1*, and in those induced by beryllium metal strikingly 80% were methylated at *Cdkn2a* and 50% at *Esr1* (Belinsky et al., 2002), suggesting a role for epigenetic alteration in the mechanisms of these particulates' carcinogenicity. A study using primary bronchial epithelial cells from non-smokers has shown dramatic alterations in miRNA expression following treatment with diesel exhaust particles (197 of 313 measured were up or down-regulated more than 1.5 fold), and suggested that the alterations were associated with inflammatory response pathways (Jardim et al., 2009). Significantly lower peripheral blood *NOS2* promoter methylation was observed in steel plant workers with exposure to particulate matter of $<10 \mu\text{m}$ (PM_{10} ; Tarantini et al., 2009). In addition, this same study reported

significantly reduced methylation of LINE-1 and Alu repeat elements in blood DNA associated with long-term PM_{10} exposure (Tarantini et al., 2009). Consistent with this, a recent report examining the affect of ambient particulate pollutants on repeat element methylation in subjects from the Boston area Normative Aging Study found significantly decreased LINE-1 methylation following recent exposure to higher black carbon (Baccarelli et al., 2009).

Ren et al. (2010) found that interquartile range increases in $\text{PM}_{2.5}$ and black carbon were associated with increases in plasma homocysteine (part of one-carbon metabolism), and glutathione (an antioxidant dependent upon homocysteine levels) transferase theta genotype. This same group of authors has also reported that increases in mean air pollution PM_{10} concentrations did not significantly alter fasting or post methionine-load total homocysteine in non-smokers, but was associated with significantly increased homocysteine levels in smokers, suggesting interactions between exposures may contribute to epigenetic alterations (Baccarelli et al., 2007). Another line of evidence for modification of exposure effects on epigenetics by genetics comes from Wilker et al. (2010) who have reported that increases in black carbon associated with increased blood pressure are modified by SNPs in miRNA processing machinery genes such as *DICER* and *GEMIN* genes.

ENDOCRINE DISRUPTORS

Exposures to endocrine disrupting chemicals such as the model toxicant diethylstilbestrol (DES) and the omnipresent bisphenol A (BPA) are of particular concern in the context of development. *In utero* exposure of mice to DES has been shown to result in the hypermethylation of the developmentally critical (specifically to uterine organogenesis) *Hoxa10* gene (Bromer et al., 2009). Although not a genotoxic agent, epidemiologic evidence from individuals exposed to DES during the first 3 months *in utero* indicates an increase in vaginal clear cell carcinoma incidence and reproductive disorders as adults (Newbold, 2004). In addition, grandchildren of DES exposed women reported higher incidences of rare reproductive disorders; whether this reflects detection bias or possibly implicates a role for epigenetic transgenerational inheritance remains to be clarified (Newbold, 2004). Ruden et al. (2005) drawing similarities between DES and Hsp90 has proposed a mechanism through which DES establishes altered epigenetic marks capable of transgenerational inheritance, wherein DES plays a role in modifying H3K4 methylation by increasing the activity of the H3K4 methyltransferase *SMYD3*, thereby altering epigenetic control of various genes.

Animal studies have demonstrated that developmental exposure to BPA can alter epigenetic profiles. In agouti mice, Dolinoy et al. (2007) showed that *in utero* BPA exposure decreases CpG methylation and that methyl-donor supplementation negated BPA related hypomethylation. In human placental cell lines, BPA exposure has been shown to alter miRNA expression levels (Avisar-Whiting et al., 2010), and specifically, miR-146a was strongly induced by BPA treatment. This resulted in both slower proliferation rate and higher sensitivity to the DNA damaging agent bleomycin (Avisar-Whiting et al., 2010). BPA, like DES, has also been shown to alter the methylation status of the *Hoxa10* gene in rodent *in utero* exposure models (Bromer et al., 2010), and breast

cancer cell lines exposed to BPA showed altered DNA methylation of specific gene promoters (Weng et al., 2010).

BENZENE

Benzene has been shown to be toxic to hematopoietic systems, is an established carcinogen (International Agency for Research on Cancer, 1999), and is known to be a cause of acute myeloid leukemia (AML) and myelodysplastic syndromes (Ye et al., 2008). Exposed to low-dose benzene, gasoline attendants and traffic officers have demonstrated significant leukocyte LINE-1 and Alu DNA hypomethylation as well as gene specific hypermethylation of *CDKN2B* and hypomethylation of *MAGE1* (Bollati et al., 2007). One preliminary study of benzene exposure using buffy coat DNA from six exposed workers and four unexposed workers have shown some evidence for relatively small changes in methylation (Zhang et al., 2010). Another preliminary study analyzed miRNA expression in seven exposed-control matched pairs and identified upregulated miR-154*, miR-487a, miR-493-3p, and miR-668 in exposed subjects (Zhang et al., 2010). Additional study of the potential epigenetic effects of benzene exposure is needed.

RADIATION

Though normally thought to work through DNA damage in the form of large deletions and in some cases point mutation (Mcdonald et al., 1995; Little, 2000; Prise et al., 2001), radiation may also be pathogenic through epigenetic alteration. Silencing of *Cdkn2a* was detected in lung tumors of rats induced by exposure to ²³⁹plutonium (Swafford et al., 1997). In human lung adenocarcinoma, *CDKN2A* methylation occurred more often in workers compared to non-worker controls of the Russian MAYAK weapons-grade plutonium plant, and the prevalence of this methylation exhibited a dose-response with radiation internal exposure dose (Belinsky et al., 2004). Methylation of *CDKN2A* has also been linked to reactive oxygen species produced by radiation exposures (Romanenko et al., 2002), and murine models of radiation-induced lymphoma have also demonstrated hypermethylation of *Cdkn2b* (encoding p15ink4b (Malumbres et al., 1997, 1999; Cleary et al., 1999)).

MECHANISMS OF TOXICANT EFFECTS ON EPIGENOMICS

Beyond effects on the one-carbon metabolism pathway, one must consider other more directed mechanisms through which toxicants could lead to somatic changes in DNA methylation, which could be clonally propagated during pathologic processes. For example altered DNA methylation may be related to increased reactive oxygen species as a result of inflammatory response or as a result of exposure to an ROS producing toxicant. It has been reported that 5-hydroxymethylcytosine can be generated by oxidation of 5-methylcytosine (Masuda et al., 1975), and both 5-methylcytosine adjacent to 8-oxoguanine, and 5-hydroxymethylcytosine have been shown to inhibit binding of methyl-CpG binding protein 2, a critical epigenetic regulator that recruits cytosine methyltransferases and histone deacetylases (Valinluck et al., 2004). It is also known that 5-hydroxymethylcytosine is not recognized as 5-methylcytosine by the maintenance methyltransferase DNMT1, and hence, may lead to aberrant loss of methylation during cell replication (Valinluck and Sowers, 2007). Additional base alterations occur via

neutrophil and eosinophil peroxidase-derived HOCl and HOBr which can react with DNA to form 5-chlorocytosine and 5-bromocytosine respectively (Henderson et al., 2003). These halogenated cytosines can be mistaken by DNMT1 as 5-methylcytosine during replication, thus providing a potential mechanism for inflammation-induced aberrant hypermethylation (Valinluck and Sowers, 2007).

Likely critical to functional epigenetic alterations important in pathology is the characteristic ability of both mutagenic and non-mutagenic compounds to impart selective pressures on cellular clones. Some researchers believe that it is this effect, and not genotoxicity, that provides the carcinogenicity attributable to some exposures, by selecting pre-existing genetic or possibly epigenetic alterations (Thilly, 2003). If *de novo* methylation in the developing tumor occurred stochastically or due to the mechanisms described above, cells with a silenced tumor suppressor may be selected for growth or expansion due to exposure to specific carcinogens. In time these pressures will allow then for clonal expansion of the epigenetically altered cells into fields which now have an increased susceptibility for tumorigenesis (Slaughter et al., 1953; Wiencke and Kelsey, 2002).

AGING

The process of aging, and differences in the environment experienced over a lifetime has been hypothesized to influence clinically significant changes in methylation profiles. Aging also provides an interesting platform to consider additional mechanisms responsible for altered DNA methylation patterns. A study of monozygotic twins demonstrated that young twin-pairs seemed more epigenetically similar than older monozygotic twins, and suggests that such drift may be at least in part responsible for differences in disease susceptibility (Fraga et al., 2005). Motivated by studies of methylation in cancer, an early report from Issa et al. (1994) described an association between aging colonic mucosa and estrogen receptor methylation. This type of research has been extended more recently to show an overall trend of increased methylation associated with older age in normal human prostate and colon tissues in several genes (Shen et al., 2005; Kwabi-Addo et al., 2007). Although an increase in promoter methylation with aging is generally accepted, and is consistent with hypermethylation of tumor suppressor genes in cancer, a disease of aging, recent evidence from Bjornsson et al. (2008) suggests a more complex picture. These authors measured intra-individual global methylation changes over >10 years and found both increased and decreased methylation levels dependent on the individual, with over 50% of participants exhibiting >5% change in methylation in peripheral blood cell DNA over time.

A study of over 200 non-pathologic human tissues from 10 anatomic sites using the GoldenGate methylation array platform (1413 autosomal CpG loci) demonstrated consistent associations between methylation and age at previously reported candidate genes, and in conjunction with other reports, suggested that the relation between aging and promoter CpG methylation is complex (Christensen et al., 2009b). The direction and strength of correlation between age and methylation were largely dependent upon CpG-island status. Specifically, a propensity for CpG-island loci to gain methylation with age, and non-island CpGs to lose

methylation with age was found. These results were consistent with (Issa, 2003; Shen et al., 2005; Kwabi-Addo et al., 2007), as well as with the findings of Tra et al. (2002) and Bjornsson et al. who showed bi-modal age-related methylation in normal tissues. Using restriction-landmark genome scanning of over 2000 CpG loci in T lymphocytes comparing newborns, middle age, and elderly people, reported that 29 loci had age-related methylation alterations, with 23 loci displaying increased methylation with age and six decreasing with age. In addition Teschendorff et al. (2010) have suggested that age-dependent methylation of polycomb-group target genes (genes suppressed in stem cells allowing differentiation) is a hallmark of cancer and is independent of gender, tissue type, or disease state, suggesting a mechanism for aging to predispose to carcinogenesis.

ADDITIONAL MECHANISTIC CONSIDERATIONS

The observed pattern of age associated methylation in (Christensen et al., 2009b) was also irrespective of tissue type, suggesting a common mechanism or dysregulation to explain these alterations. Langevin et al. (2011) expanded these analyses to the larger, Illumina Infinium Methylation27 platform, assessing nearly 27,000 autosomal CpG loci in peripheral blood samples of healthy adults, and found again that the direction and magnitude of the association between methylation at CpG site and age was influenced by local sequence features. In addition, the study from Christensen et al. (2009b) that examined non-pathologic tissue methylation assessed different exposure types in different tissues. Though a detailed analysis that included genomic context was not published, these authors observed a consistent trend for exposure-related methylation alterations across tissue types. Assessment of pleural tissue methylation by asbestos exposure history, lung tissue by smoking history, and peripheral blood methylation by alcohol intake each indicated that exposure-associated methylation alterations were dependent on genomic context for CpGs analyzed. However, unlike age-related methylation alterations (increased methylation of CpG-island loci with age, and decreased methylation of non-CpG-island loci with age) exposure-related methylation alterations each had a contrasting pattern where CpG-island loci decreased with exposure, and non-CpG-island loci had increased methylation with exposure (unpublished). These reports highlight the importance of recognizing that contextual elements may differentially affect the likelihood and direction of exposure-related epigenetic alterations. Though these findings suggest that different mechanisms are responsible for age-related and exposure-related methylation alterations, and that some exposures may share common mechanisms for altering methylation, additional population-based research on appropriate target tissues is necessary to advance our understanding of any shared (within and across exposure types) or disparate mechanisms responsible for methylation alterations.

Beyond sequence context, one may also consider the chromatin environment to understand regions targeted for alterations of DNA methylation. Rakyen et al. (2010) showed that aging-associated hypermethylation was more likely to occur at promoters associated with bivalent chromatin domains. Additionally, distinct mechanisms may be responsible for altered repeat element (global) methylation and altered promoter methylation.

Although traditional dogma indicates separate methyltransferase enzymes for maintenance (*DNMT1*) and *de novo* methylation (*DNMT3A/B*), a recent perspective from Jones and Liang (2009) proposes that maintenance methylation in CpG-islands may be a cooperative process. When considering the mechanisms of environmental exposure-related methylation alterations it is important to remain conscious of the fact that we do not have a complete understanding of *de novo* and maintenance methylation mechanisms. Reduced fidelity of maintenance methyltransferases with aging is one potential explanation for age-related decreases in methylation; while age-related increases in methylation could potentially reflect the accumulation of stochastic methylation events over time. Another mechanism that has been hypothesized is a spreading process, whereby CpG methylation from within the gene migrates into the promoter due to the loss of protective boundary elements which normally protect promoters from CpG-island methylation (Turker and Bestor, 1997; Sun and Elgin, 1999; Turker, 2002). Alternatively, for example in aging, a spreading process subsequent to a stochastic methylation alteration (in pathologically normal tissue) without functional consequences for gene expression, may allow the initiation of a spreading process that eventually confers an altered expression phenotype. Yet, these mechanisms do not fully explain the apparently sequence context specificity of these observations, thereby suggesting that the age-related alterations of methylation observed may be influenced in part by targeted mechanisms that have yet to be fully explained. Using long-term culture of mesenchymal stem cells, Bork et al. (2010) show that for the majority of CpGs examined (using the Illumina Infinium Methylation27 platform), the methylation levels remain constant, and that only a specific set of CpGs undergo differential methylation with long-term culture, and that these sites were over-represented by homeobox genes. This same group (Koch et al., 2011) demonstrated similar findings in aging dermal fibroblasts, although often in directions opposite those observed in the MSCs. Such results suggest that aging related DNA methylation may be following a developmental program or resulting from the loss of some developmentally regulated maintenance.

IMPORTANCE OF EPIGENOMICS IN ENVIRONMENTAL HEALTH

We have highlighted what is a growing body of literature beginning to link epigenetic alterations to toxicant exposures from *in vitro* work, animal models, and human observational studies. Toxicologists and environmental health scientists, in addition to studying the effects of exposures at the genotoxic and transcriptional levels, must also consider a toxicant's effect on the epigenome, a task which requires multi-disciplinary work from *in vitro* and model systems to epidemiologic studies of human disease. New technologies now allow for global analysis of epigenetic alterations and these may provide insight into the extent and patterns of alteration in normal and diseased tissue. Notably, the latest generation of CpG methylation arrays interrogates hundreds of thousands of CpG loci, includes all designable RefSeq genes, and is low-cost enough for application in population-level studies. Next generation sequencing approaches, as their cost decreases and throughput increases will also become increasingly used as tools for annotating the epigenome and its relation to exposures. Appropriate

models must also be considered, and may include mammalian stem cells which could prove extremely useful in bettering the understanding of epigenetics in development and beyond. Finally, epidemiologist must begin to consider how to incorporate examinations of the epigenome in population-based studies, in order to define consistent methodologies and data collection that is crucial to understanding the true effect of environmental exposures on the human epigenome. Although examinations of epigenetic alterations related to exposures are emerging it is still important to note that the mechanisms by which toxicants and the environment modulate the epigenetic landscape of individual cells are

yet to be elucidated and require further exploration. This work is urgently needed in order to better understand the biology of epigenetic alterations and the effects of toxic exposures on these disease-associated somatic epigenetic alterations. Better defined mechanisms will lead to better prediction of the toxic potential of new compounds introduced into the environment, and allow for more targeted and appropriate disease prevention strategies.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 October 2011; accepted: 04 November 2011; published online: 22 November 2011.

Citation: Christensen BC and Marsit CJ (2011) Epigenomics in environmental health. *Front. Genet.* 2:84. doi: 10.3389/fgene.2011.00084

This article was submitted to *Frontiers in Toxicogenomics*, a specialty of *Frontiers in Genetics*.

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