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Epigenetics, oxidative stress and Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose clinical manifestations appear in old age. The sporadic nature of 90% of AD cases, the differential susceptibility and course of illness, as well as the late age onset of the disease suggest that epigenetic and environmental components play a role in the etiology of late onset AD (LOAD). Animal exposure studies demonstrated that AD may begin early in life and may involve the interplay between the environment, epigenetics and oxidative stress. Early life exposure of rodents and primates to the xenobiotic metal lead (Pb) enhanced the expression of genes associated with AD, repressed the expression of others, and increased the burden of oxidative DNA damage in the aged brain. Epigenetic mechanisms that control gene expression and promote the accumulation of oxidative DNA damage are mediated through alterations in the methylation or oxidation of CpG dinucleotides. We found that environmental influences occurring during brain development inhibit DNA methyltransferases, thus hypomethylating promoters of genes associated with AD such as the beta- amyloid precursor protein (APP). This early life imprint was sustained and triggered later in life to increase the levels of APP and amyloid-beta (Aβ). Increased Aβ levels promoted the production of reactive oxygen species (ROS) which damage DNA and accelerate neurodegenerative events. While AD-associated genes were over-expressed late in life, others were repressed, suggesting that these early life perturbations result in hypomethylation as well as hypermethylation of genes. The hypermethylated genes are rendered susceptible to Aβ-enhanced oxidative DNA damage because methylcytosines restrict repair of adjacent hydroxyguanosines. While the conditions leading to early life hypo or hyper methylation of specific genes are not known, these changes can impact gene expression and imprint susceptibility to oxidative DNA damage in the aged brain.

Keywords

epigenetics; DNA methylation; DNA oxidation; APP; amyloid; Alzheimer's disease; Pb exposure

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Introduction

Epigenetics is a major mechanism that accommodates gene-expression changes in response to gene-environment interactions [1,2]. Epigenetics refers to modifications in gene expression that are influenced by DNA methylation and/or chromatin structure, RNA editing and RNA interference without any changes in DNA sequences [3]. DNA methylation and histone deacetylation are known to occur shortly after DNA synthesis and could be modified by diverse physiological or pathological factors altering gene expression for the lifetime of the organism.

Epigenetics and mental disorders

Despite that the bulk of the work in epigenetics has been developed in cancer research [4], there is recognition of epigenetic aberrations in mental illnesses, namely in fragile X disease and Retts syndrome [5–7]. Fragile X disease is associated with an expanded (>250 copies) number of hypermethylated CGG repeats 5′ of the FMR1 gene that results in down regulation of the gene [5,6]. Disease severity in Fragile X is directly correlated with the extent of methylation in the 5′ region of the FMR1 gene [8]. Retts syndrome, on the other hand, is linked to mutations in the gene encoding the methylated cytosine binding protein (MECP2) [7]. The MECP2 recruits a variety of proteins that form a complex thus repressing gene expression [9]. Both, Fragile X and Retts syndrome, are responses to well established alterations to a single gene. However, recent work in autism spectrum disorders suggests a major epigenetic component to the origin of the disease, indicating some contribution of differential methylation, but not to a single gene [10].

DNA methylation is one of the most studied aspects of epigenetic modifications. The addition or removal of methyl groups from cytosines can impact gene expression and alter cell and organism function. For example, hypomethylation of the membrane-bound catechol-O-methyltransferase (MB-COMT) gene has been implicated in both schizophrenia and bipolar disorder [11]. On the other hand, hypermethylation of the RELN (reelin) gene has been shown to be associated with schizophrenia [12]. In addition to DNA methylation, other mechanisms linked to epigenetic regulation have been found to play a role in neuronal function, as demonstrated by the use of inhibitors of histone deacetylases to ameliorate deficits in a wide range of psychiatric and neurological conditions [13]. Recent research points to endogenous systems, such as sirtuins, a group of nicotinamide (NAD+)-dependent deacetylases/ADP-ribosyltransferases, playing a major role in the beneficial response to longevity extending protocols such as caloric restriction [14]; however, whether their neuroprotective effects are due to the deacetylase activity remains to be elucidated [15].

Genetics and epigentics of AD

Alzheimer's disease (AD) is a gradual and irreversible, progressive neurodegenerative disorder which results in dementia and death. AD pathology is characterized by senile plaques and neurofibrillary tangles (NFTs), combined with massive neuronal loss, mainly in the hippocampus and association regions of the neocortex. The major constituents of senile plaques are 39–42 amino acid peptides, snipped from a larger protein called beta-amyloid precursor protein (APP) [16–21]. Of these, the Amyloid-β (Aβ) form that is comprised of 42 amino acids is considered the most amyloidogenic.

The majority of AD cases occur in the elderly; however, it is still unresolved whether AD is a disease of old age or whether it has earlier beginnings. Epidemiological studies have shown that people with dementia are more likely to have had low scores on intelligence tests when they were children when compared to people without dementia [22]. These findings indicate that AD patients may arrive at old age with significant predisposing deficits. Some studies point to a possible role for epigenetic changes on AD etiology. AD patients are among the few

that may display high homocysteine (HCY) and low B12 and folate in blood, suggesting a dysregulation in the s-adenosylmethionine cycle required for epigenetic regulation through DNA methylation. It is worth noting that expression of APP and beta-APP cleaving enzyme (BACE) genes is regulated via methylation of their promoters [23].

Furthering the possibility for an epigenetic impact in AD, structural genomics studies have demonstrated that more than 200 genes might be involved in AD pathogenesis [24]. In addition, the AD population exhibits a higher absolute genetic variation rate of 40–60% and AD patients differ in their genomic architecture from patients with other forms of dementia [24]. Between 5–10% of AD cases are of familial origin and involve mutations in genes associated with APP biosynthesis and proteolytic processing [25–28]. The genetics of AD have revealed that early (<60 years old) onset AD (EOAD) is associated with APP or the presenilins, while the risk to develop late onset AD (LOAD) is linked to the apolipoprotein E (ApoE) polymorphism [29].

Genetics plays a major role in EOAD; however, LOAD which represents over 90% of cases is sporadic in nature and remains with an unexplained etiology. Twin studies often used to confirm the inheritance pattern of a disease have shown an estimated concordance well below 100% (20–80%) for AD, suggesting that LOAD is a complex non-Mendelian disease [30–32]. Prefrontal cortex and lymphocytes from AD patients were used to analyze DNA methylation patterns in genes with a potential role in AD etiology. An age-specific epigenetic drift associated with unusual methylation patters in LOAD was identified, supporting a potential role of epigenetic effects in the development of the disease [33]. Additionally, genes that are genetically associated with LOAD (PSEN1, APOE) showed the largest inter-individual variance in DNA methylation, with the APOE gene exhibiting the most variably methylated sequences. APOE presented a bimodal methylation pattern, with a hypomethylated CpG-poor promoter and a fully methylated 3′-CpG-island, containing the sequences for the ε4-haplotype, the only established genetic risk factor for LOAD [33]. Interestingly, the gene MTHFR, coding for methylenetetrahydrofolate reductase, showed a significant inter-individual epigenetic variability. Alteration in MTHFR expression can influence homocystine levels, which may contribute to LOAD predisposition [33]. The sporadic nature of the disease, the differential susceptibility and course of illness in males and females, as well as the late age onset of the disease add to the hypothesis that epigenetic and perhaps environmental components play a role in the etiology of LOAD [33].

Role of oxidative stress in the etiology of AD

In addition to the established pathology of amyloid plaques and neurofibrillary tangles in the brain of AD sufferers, there is a growing body of evidence indicating changes in the redox status of AD brains. This is supported by findings of increased levels of oxidative damage markers in every major cellular macromolecule (proteins, lipids, and DNA) [34]. Also, alteration in the expression of antioxidant systems lends support to a role for free radical damage in AD pathology [35]. Due to their postmitotic nature, damage to DNA in neurons could be highly detrimental to their function and viability. Guanine (G) has the lowest oxidation potential of the DNA bases; thus, 8-oxo-7,8-dihydro-2′-deoxyguanosine (oxo^8dG) is the most prevalent form of oxidative base modifications produced [36–38]. Some evidence suggests that in addition to its mutagenic properties, presence of 8-oxodG in DNA can alter binding of transcription factors and can impact epigenetic signaling [39,40].

Scope of the present review

This review focuses on epigenetics and explores the role of the environment in the promotion of AD pathogenesis through transcriptional dysregulation of genes associated with AD. In addition to the alteration in APP and Aβ metabolism, age-related accumulation of oxidative damage is also suspected to play a role in the pathogenesis of AD. Thus, any environmental

agent that significantly alters the redox potential of the aging brain can theoretically promote AD pathology. Here we will explore the interactions between DNA methylation and DNA oxidation and propose mechanisms that attempt to explain LOAD as a disease in which gene expression is reprogrammed by early life events that alter the methylation profile of genes and modify susceptibility to DNA damage thus resulting in neurodegeneration.

Exposure to Pb and the developmental-basis of AD

In a seminal study in 1989, David Barker and colleagues demonstrated an inverse relationship between birth weight and the incidence of cardiovascular disease. The Barker hypothesis, also known as the Fetal or "Developmental Basis of Adult Disease", states that many adult diseases might have a developmental origin [41–44]. A large body of subsequent clinical and experimental data has supported this hypothesis and has shown that diseases of the cardiovascular system, hypothalamic–pituitary–adrenal (HPA) axis, and diabetes can also be affected by nutritional imbalances during pregnancy [45–47]. Some clinical and animal studies suggest that certain deficiencies in the CNS in adults may also be rooted in alterations during development. Memory impairment in adult animals and diseases such as schizophrenia have been linked to infection, fetal malnutrition or hypoxia in early life [48–51]. In addition to lending support for a developmental origin of disease, these observations serve to propose a new concept regarding some adult diseases that emphasizes the role of environmental factors acting in the periconceptual, embryonic, fetal, and infantile phases of life [52]. The impact of such influences early in life maybe partially mediated through epigenetic mechanisms that involve DNA methylation.

Given the recognized effects of Pb exposure in children, important work has been directed to understand the consequences and the mechanisms linked to Pb exposure in this susceptible population [53]. Pb is known to produce cognitive and behavioral deficits in children [54,55] with the added risk of being ubiquitous in distribution. In relation to risks associated with Pb exposure in adult populations, several population-based case-control studies have found that chronic occupational exposure to Pb as well as other metals is associated with the incidence of Parkinson's disease [56]. Other studies point to a relation between high blood and bone Pb levels and increased risk of Amyotrophic Lateral Sclerosis (ALS), suggesting that Pb exposure plays a role in the etiology of the disease [57]. While these studies provided hints as to the possible connection between Pb exposure and neurodegenerative disease, a seminal work looked at tibia bone Pb levels in 529 former organo-lead workers and its relationship to ApoE genotype, a known risk factor for AD. Results led to conclusion that the persistent CNS effects of Pb are more toxic in individuals with at least one ApoE ε4 allele [58]. The link between past adult Pb exposure and neurodegeneration was further established by the same research group using brain MRI imaging [59] and was consistent with their previous work showing an association between Pb exposure and longitudinal cognitive decline. While these studies focused on adult occupational exposure to Pb, it is not known if the workers they studied had been previously exposed to Pb as children.

Animal studies from our laboratories strongly suggest that exposure to Pb during development can be a risk factor that promotes the pathogenesis of AD [60]. To examine latent responses to developmental Pb exposure, we monitored the lifetime expression of the APP gene. We observed that APP mRNA expression was transiently-induced in neonates, but exhibited a delayed over-expression 20 months after exposure to Pb had ceased. This up-regulation in APP mRNA expression was proportionate with a rise in activity of the transcription factor Sp1 (specificity protein 1), one of the regulators of the APP gene. Furthermore, the increase in APP gene expression in old age was accompanied by an elevation in APP and its amyloidogenic Aβ product. However, APP gene expression, Sp1 activity and APP and Aβ protein levels were unresponsive to Pb exposure during old age.

In order to link these molecular perturbations to pathological consequences associated with AD, we acquired the brains of cynomolgus monkeys who have been similarly exposed to Pb as infants in the 1980's. Primates are among the few animal models that express amyloid plaques and other pathological features that are absent in normal non-transgenic rodents. Experiments in tissue derived from these primates demonstrate that the APP mRNA, APP, and Aβ are elevated in old monkeys developmentally-exposed to Pb. Immunohistochemical analysis of Aβ deposition shows that early exposure to Pb alters the distribution of intracellular Aβ staining and plaques formation [61]. These data suggest that environmental influences occurring during brain development pre-determined the expression and regulation of APP later in life, potentially altering the course of amyloidogenesis [60].

Ample evidence has accumulated that oxidative damage to macromolecules such as DNA, protein, and lipids, as well as, a down-regulation in antioxidant enzymes is associated with AD [62–65]. Interestingly, we have found elevations in the oxidative DNA marker 8-oxo-dG in older rats that had been developmentally exposed to Pb [66]. We also found a similar accumulation in 8-oxo-dG in aged primates developmentally-exposed to Pb [61]. There are alternative hypothesis to elucidate the origin of this latent buildup of oxidized DNA. Increases in Aβ could lead to the generation of reactive oxygen species promoting the formation of 8-oxo-dG; alternatively, epigenetic modulation in the methylation pattern of cytosines could interfere with the repair or oxidation potential of adjacent oxidized guanines [67].

DNA methylation and the environment

The late onset responses to developmental exposure to Pb are probably unique to the nervous system and few other tissues. Since cells in most tissues turn-over and proteins are in a continual cycle of synthesis and degradation, the molecular targets that would store and transmit this information would have to reside in the genome of terminally-differentiated cells or cells that continually divide passing along their genetic makeup. Furthermore, for the damage to persist on the structure of the DNA, the perturbation has to escape recognition by DNA repair enzymes.

DNA methylation of cytosines is a major epigenetic event that can influence the regulation of gene expression and has been linked to the process of gene imprinting in mammals. Alterations in 5-methylcytosine patterns on the promoters of genes are the first level of regulation of gene expression in development, differentiation, carcinogenesis, and aging. The methylation of cytosine can sometimes serve as a heritable code by the selective action of some methylases that act on cytosine nucleotides in a CG sequence. Methylation occurring predominantly at this symmetrical CG dinucleotide, due to the preferences of DNA methyltransferase for a hemi-methylated substrate, maintains specific heritable patterns of methylation [68].

DNA methylation patterns are mainly established *in utero*, and it has been established that the fetal environment may alter such patterns leading to sustainable changes in gene expression that endure for a lifetime [69]. In terms of the brain, this environmentally-dependent modulatory period may continue into postnatal development. Genome-wide demethylation patterns are also observed shortly after fertilization and followed later by a new wave of methylation of the CG sequences [70]. Thus, the process of methylation and demethylation appears to be a controlled programmed event providing cells with a broad developmental potential and a mechanism that widens the means to regulate the expression of genes and to transmit information beyond the one stored in the genetic code.

Animal studies have clearly demonstrated that early-age environmental stimuli can alter methylation patterns leading to gene expression changes which may result in modified behaviors or increase disease risk in adulthood. It was shown that maternal grooming changed the methylation pattern and expression of the glucocorticoid receptor in the hippocampus in rat offspring, leading to permanent changes in their stress response [71]. In a different approach,

it was shown that modification of the maternal diet through pregnancy led to a decrease in the methylation of the glucocorticoid receptor (GR) and the peroxisomal proliferator-activated receptor (PPAR) genes which was consistent with their elevated mRNA expression in the offspring after weaning [72].

In addition to behavioral and nutritional imbalances, chemical exposure can also interfere with the status of DNA methylation. One way in which environmental agents or occupational exposure could interfere with DNA methylation is by disrupting the enzymes that conduct such reactions. In vitro studies show that the addition of cadmium (Cd) to hepatic nuclear extracts inhibited DNA-methyltransferase [73]. More recently, it was reported that subchronic exposure to Cd inhibited DNA-methyltransferase activity in cultured cells, while chronic exposure enhanced the activity of the DNA-methyltransferase. The Cd effect in DNA methyltransferase translated into altered levels of methylation of DNA, suggesting that the action of Cd on DNA methylation may be responsible for its carcinogenic properties [74]. Some studies suggest that modifications in methylation patterns might be related to other epigenetic processes such as histone modifications [75]. In addition to DNA-methylation, environmental agents could also disrupt chromatin restructuring and produce long-term alterations in gene expression. Reports that examined sperm chromatin structure in monkeys found alterations at environmentally-relevant blood Pb levels and other studies have shown decreases in the level of protamine-DNA interactions that may alter sperm chromatin condensation [76,77]. However, very few studies have been conducted on DNA-methylation in the brain and none have examined the potential of environmental agents to disturb this process.

Structure of the APP promoter and DNA Methylation

The GC content of the APP promoter is estimated to be 72%, and the rate of CpG dinucleotides is five times that observed in other eukaryotic promoters indicating that its expression would be subject to regulation by DNA-methylation [78–82]. The Sp1 consensus sequence, 5′ GGGCGGG (lower strand, 5′CCCGCCC) contains CG dinucleotides and is present in several places on the APP promoter. Few studies have examined methylcytosine levels on the APP promoter and the published work provides a varied picture depending on the region of the promoter that was examined. An analysis of CpG elements in the APP promoter region between −460 and −275 did not detect methylation of cytosines in healthy human brain tissue [83]. On the other hand, it was found that regions of the human and primate APP promoter upstream of −500 displayed tissue and brain region-specific profiles of methylation, which crudely reflect APP expression patterns [84]. More recently, it was established that there are at least 13 potential methylation sites in the region −236 to −101 of the human APP promoter [85,86]. Twenty-six percent of these cytosines were more frequently methylated in healthy individuals between the ages of 35–70 years old, as compared to 8% in those aged 74–90 years of age. This age–related reduction in methylcytosine was more prominent (ten-fold reduction) in some locations (−207 to −182) of the APP promoter that belong to 9- and 11-bp-long GC-rich elements, which are typical Sp1 DNA-binding sites [85]. Furthermore, these investigators suggested that age-related demethylation of cytosines may have some significance in the Aβ deposition in the aged brain [85]. Interestingly, BACE (beta-secretase), as well as PS1, are also regulated by methylation, translating into changes of Aβ levels [87].

It is plausible that developmental exposure to Pb could exacerbate the demethylation process of the APP promoter in old age thus elevating APP expression. Studies have shown that Sp1 DNA-binding is inhibited by methylation of cytosine in proximity to CpG sites in the consensus sequence [88,89]. The expression of Sp1 target genes such as the epithelial gene T1a and MAO B have also been shown to be regulated by increased methylation of CpG sites on or around Sp1 DNA-binding sites [90–92].

DNA methylation and oxidative stress

In CpG dinucleotides, the cytosine is the preferred base for DNA methylation, while the guanine is the site for oxidative damage. 8-oxo-dG is widely used as biomarker of oxidative DNA damage. In the absence of exogenous DNA-damaging reagents, endogenously formed metabolic reactive oxygen species (ROS) are able to create 10^5 molecules of 8-oxo-dG in the cells per day [93]. Oxidative DNA damage is primarily repaired by the base excision pathway and base excision repair is initiated by a DNA glycosylase that recognizes the modified base [94]. Oxoguanosine DNA glycosylase 1 (OGG1) is the major repair enzyme to recognize and remove 8-oxo-dG. Oxidative DNA damage and possible deficiencies in Ogg1 are considered to be a central factor in the process of aging and aging-related diseases such as Alzheimer's disease [95,96].

Our study has shown that developmental Pb exposure increases Aβ levels as well as 8-oxo-dG production in old age [66]. Aβ is known to induce functional disturbances in vivo through its pro-oxidant and neurotoxic properties [97,98]. Aβ promotes the formation of reactive oxygen species (ROS) and the use of antioxidant can prevent Aβ elicited neurotoxic cascades [99–102].

Few studies have addressed DNA methylation and DNA oxidative damage simultaneously as an epigenetic phenomenon, and little is known on how DNA methylation and DNA oxidation interact with each other. Researchers using synthetic DNA oligonucleotides have found oxidation of guanine in CpG dinucleotide reduced the MBD (methyl group binding domain) binding to that site [40]. When 5-methylcytosine is oxidized to 5-hydroxymethylcytosine, its affinity to MBD is greatly reduced to the same level as unmethylated cytosine. Likewise, 8-oxo-dG inhibits adjacent cytosine methylation [103,104]. Methylated CpG has also been found to account for decreased transcription factor binding to the promoter region [89,91].

Given evidence that methylation regulates gene expression, results from our Pb exposure studies suggest the occurrence of both hypomethylation and hypermethylation of genes. mRNA levels of a series of transcription factors (TFs) were screened on postnatal day 5. This short term exposure to Pb resulted in changes in the signal-dependent TFs such as the steroid receptor superfamily (GR, ER, PR, RXRs, RARs, etc.), and development-specific factors (Sp1) [60]. The expression levels of some these TFs such as Sp1 were elevated, while the steroid receptor super family exhibited a down regulation trend. Microarray array analysis of about 588 neurobiology-related human genes was also conducted to identify the genes that are altered due to infantile exposure to Pb in the frontal association cortex of 23-year old cynomolgus monkeys (unpublished data). The results showed that the expression profile of only a few genes (22) was changed due to early life exposure to Pb. Most of the altered genes belonged to neurotransmitter, growth receptors and signal transduction pathways. Searches were conducted on various databases (Ensembl and NCBI nucleotides) to determine if the regulatory regions of these genes were rich in CpG dinucleotides (>60%). We found all of the altered genes (with the exception of two) were abundant in CpG dinucleotides in their 5′ untranslated regions (5′ UTR). About a third of these genes were elevated, while a majority were repressed.

These findings and published results on AD-related genes [60,61] demonstrate that early life exposure to Pb may enhance the expression of some genes and depress the expression of others ([58]; unpublished studies). Although the direction of change in gene expression was opposite, the modified genes have one thing in common, and that is abundance in CpG dinucleotides in their promoter regions. It is thus clear that exposure to Pb can interfere with regulation of gene expression by hypomethylating certain genes and hypermethylating others. Alteration in the methylation patterns of genes can become permanent and carry with them built in susceptibility to neurodegenerative events.

Sustainable hypomethylation of the APP promoter for example can increase the ceiling of expression of the APP gene in response to aging processes driving overproduction of APP and Aβ levels. The increased Aβ levels then facilitate ROS production with their pro-oxidant properties, damaging the DNA. Therefore, in cases of hypomethylation, DNA damage is increased from the products of over expressed pro-oxidant genes. On the other hand, hypermethylation early in life would render detrimental effects on both gene transcription and repair pathways.

To model the interactions between methylation and oxidation, we synthesized oligonucleotides that resembled the binding site for the transcription factor Sp1. In some oligos the cytosine in a CpG was replaced by methylcytosine and the guanine by an 8-oxo-dG. A third oligo containing both methylcytosine and 8-oxo-dG adjacent to each other was also prepared. We then conducted DNA-binding and repair studies. Expectedly, we found that presence of either 5-methylctosine or 8-oxo-dG dramatically suppressed Sp1 DNA-binding; however the combination of both had an effect greater than either alone (Figure 1A). Likewise, the repair of 8-oxo-dG was greatly diminished when the 8-oxo-dG was preceded by 5-methylcytosine (Figure 1B). These experiments show that the methylation status of a gene can greatly impact gene expression and DNA repair. If a gene is methylated in a regulatory region during development and faces subsequent oxidative stress in this same area, it will be greatly repressed. Likewise, such a gene will be inadequately repaired. On the other hand, oxidized DNA will inhibit DNA methylation of an adjacent cytosine [103,104].

While substitutions in synthetic DNA oligonucleotides show the interplay between oxidative damage and methylation of DNA, this relationship can also be seen in cells. Studies with oxidant-transformed cell lines have shown unusual changes of methylation patterns of several genes. This suggests the oxidative DNA damage and DNA methylation interact with each other, which may consequently alter the methylation patterns and transcriptional activity of affected genes. In the case of the APP gene, oxidative and methylating changes in its promoter regions can determine its expression as well as the levels of its gene products and their derivatives associated with amyloid formation. Both hypermethylation and hypomethylation can be impacted by environmental factors and the diet, as is oxidative stress. Through the methylation pathway, supplementation with apple juice has been shown to attenuate presenilin-1 overexpression during dietary and genetically-induced oxidative stress [105]. Indeed, fruit and vegetables juices play a beneficial role in potentially delaying the onset of Alzheimer's disease [106].

In considering methylation and oxidation, it is important to make some important distinctions. Oxidation is a dynamic process that can occur at any time there is oxidative stress but is typically high early and late in life, because high metabolic rate in the former and reduced antioxidant defenses in the later. Furthermore, these observations are based on our published work which profiled oxidative stress across the lifespan. We found that periods of early and late life are times in which ROS levels are high as well the molecular targets for them. [66]. Methylation on the other hand is poorly understood and is presumed to occur during early development and sustained for the rest of life. While there are DNA methylating enzymes, the existence of a demethylating enzyme in mammalian systems remains controversial, and actually it has been suggested that DNA repair enzymes could function a DNA demethylation role [107,108]. Despite the known fact that methyl group donors available in the diet can alter the methylation pattern in favor of hypermethylation, the natural mechanisms that underlie hypomethylation are not fully understood. Furthermore, it is important to recognize that even if a substance is not a pro-oxidant, it can still lead to an accumulation of oxidative damage through alterations in methylation patterns that impact the repair of adjacent oxidized guanines.

In our model, environmental agents such as heavy metals can inhibit the enzymes that maintain or methylate DNA. In this scenario, the exposure to such metals has to occur during a developmental period when such methylation patterns are being established; however, while it is assumed that methylation patterns are maintained for life, it is also plausible that they are dynamically changing. To evaluate this possibility, we quantified the content of methylcytosine on three positions of the APP promoter in the frontal cortex of monkeys that were 6, 12, and 23 years old. We found that they all maintained their methylcytosine content, with the exception of one position which decreased with age (Figure 2). Although this experiment examined only a few sites out of possibly thousands, it suggests that both stable methylcytosine patterns and alternating content are possible; however, how such selective effects are imparted is still unknown. In terms of its relevance in human setting, larger–scale work on human subjects supports our hypothesis by having shown that DNA methylation is altered over time in individual humans in both genomic DNA as a whole and in specific, selected gene sequences [109].

Conclusion

The Pb exposure model was used to test the hypothesis that the origins of AD begin early in life and that environmental exposure can determine future disease susceptibility. The effects of the original exposure would remain latent until an additional trigger or triggers affect the exposed organism. Such a model operates through the regulatory sequences of a gene and places the epigenotype center–stage over genotype in development of neuropsychiatric disorders. We refer to the model as a 'Latent Early–life Associated Regulation' (LEARn) model [66,110]. This model is not restricted to Pb and can be applied to nutritional deficiencies, stress, chemical exposure or any other perturbation that interferes in the epigenetic programming of gene expression. In summary our model proposes that interference by Pb early in life has a dual effect on methylation of genes in terminally differentiated neurons. Genes whose promoters are hypomethylated such as the APP gene are programmed to over-express themselves in the face of a later, secondary trigger. On the other hand, genes that are hypermethylated are destined for repression. In the context of AD pathogenesis, we hypothesize that normal aging triggers induce pathogenically high APP gene expression in exposed, hypomethylated APP, individuals, which in turn produces more APP, which is further cleaved to build up Aβ levels. Large–scale age–related change in gene expression levels has been previously documented in human brains [111]. The buildup in Aβ has multiple consequences. It can produce disruption of synaptic function as a diffusible ligand; it can aggregate to form plaques, or it can promote ROS production. ROS can in turn damage DNA and thus genes which had been previously hypermethylated will have a reduced capacity to defend and repair against this ROS onslaught because of structural alteration on CpG dinucleotides. The reduced capacity to repair DNA and the transcriptional enhancement in Aβ production converge to exact greater damage on neurons and result in cell loss. This is a hypothetical model of how early events can build in susceptibility to disease later in life via an interaction between exposure, epigenetics, and oxidative stress. Figure 3 describes the proposed model of epigenetic changes and increased susceptibility to 8-oxo-dG accumulation seen after developmental Pb exposure and in AD.

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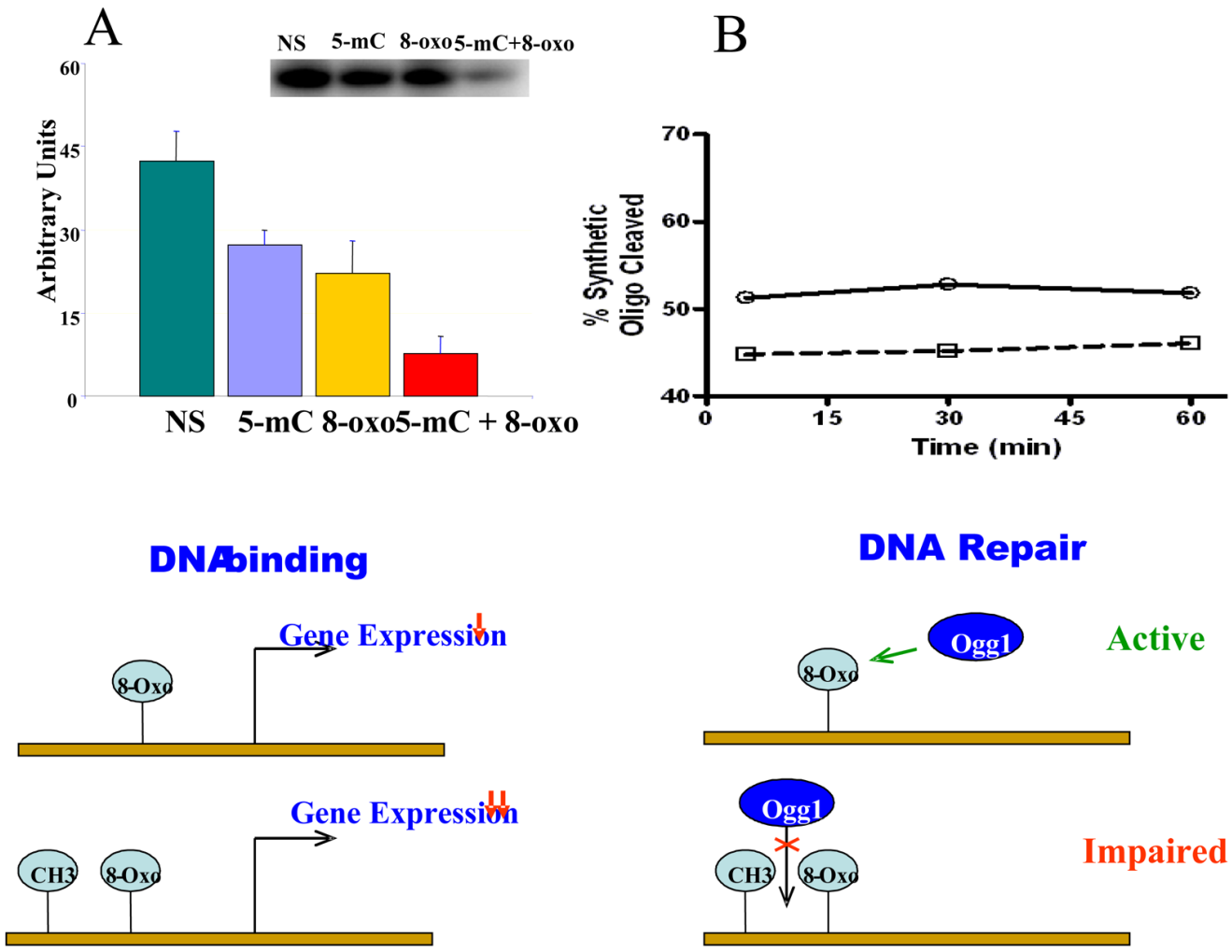

Figure 1. Structural modifications in CpG dinucleotides and their impact on Sp1-binding and DNA Repair

Sp1 binding and DNA repair glycosylase (Ogg1) activities were evaluated in oligonucleotides containing a 5-methyl cytosine and/or an adjacent 8-oxo-dG. An unmodified oligonucleotide was used as control. Top panels illustrate results for assessments of Sp1 binding (A) or Ogg1 activity (B); bottom panels illustrate the biological consequences of these modifications. A) Sp1 binding was reduced by methylation or by presence of an oxidized G. However, the inhibition was larger when both base modifications were present in the same sequence. B) Similarly, presence of a methylated cytosine next to an oxidized G reduced the activity of Ogg1, preventing repair of oxidative damage to DNA. The solid line represents time-associated repair when only 8-oxo-dG is present; while, the dashed line represents repair when 5-mC is adjacent to the oxidized G. Methods for Sp1 binding and Ogg1 activity have previously been published [60,66]. The values presented were derived from 3–4 experiments.

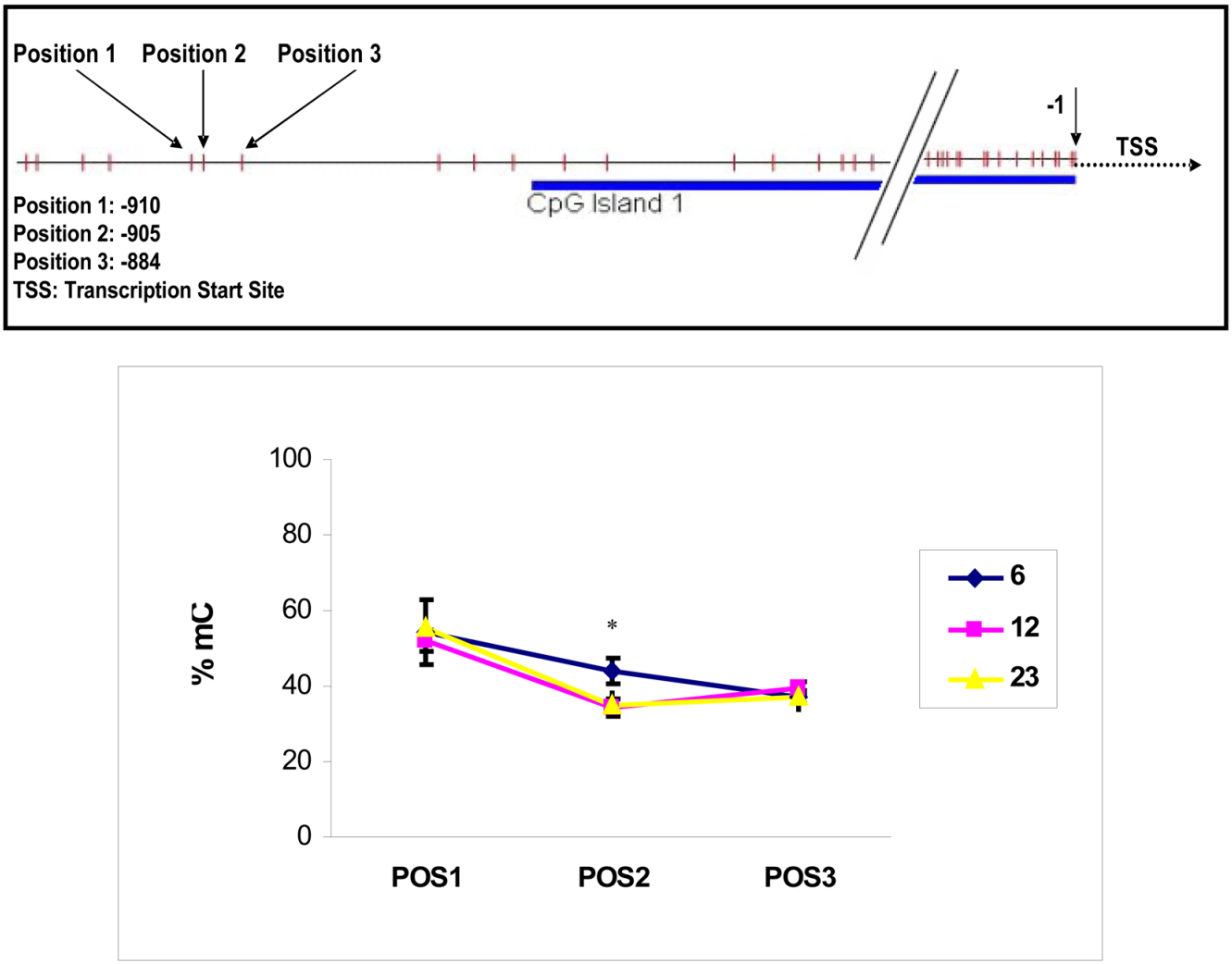

Figure 2. DNA methylation of the APP promoter across the lifespan

Pyrosequencing was used in order to quantify the methylation levels at different CpG sites of the APP promoter. The methylation levels of the CpG dinucleotides located in the illustrated region were quantified after bisulfite conversion. The graph shows changes in methylcytosine content in these three different sites at different ages in the frontal cortex of monkeys. Three monkeys per age group were used for this sequencing.

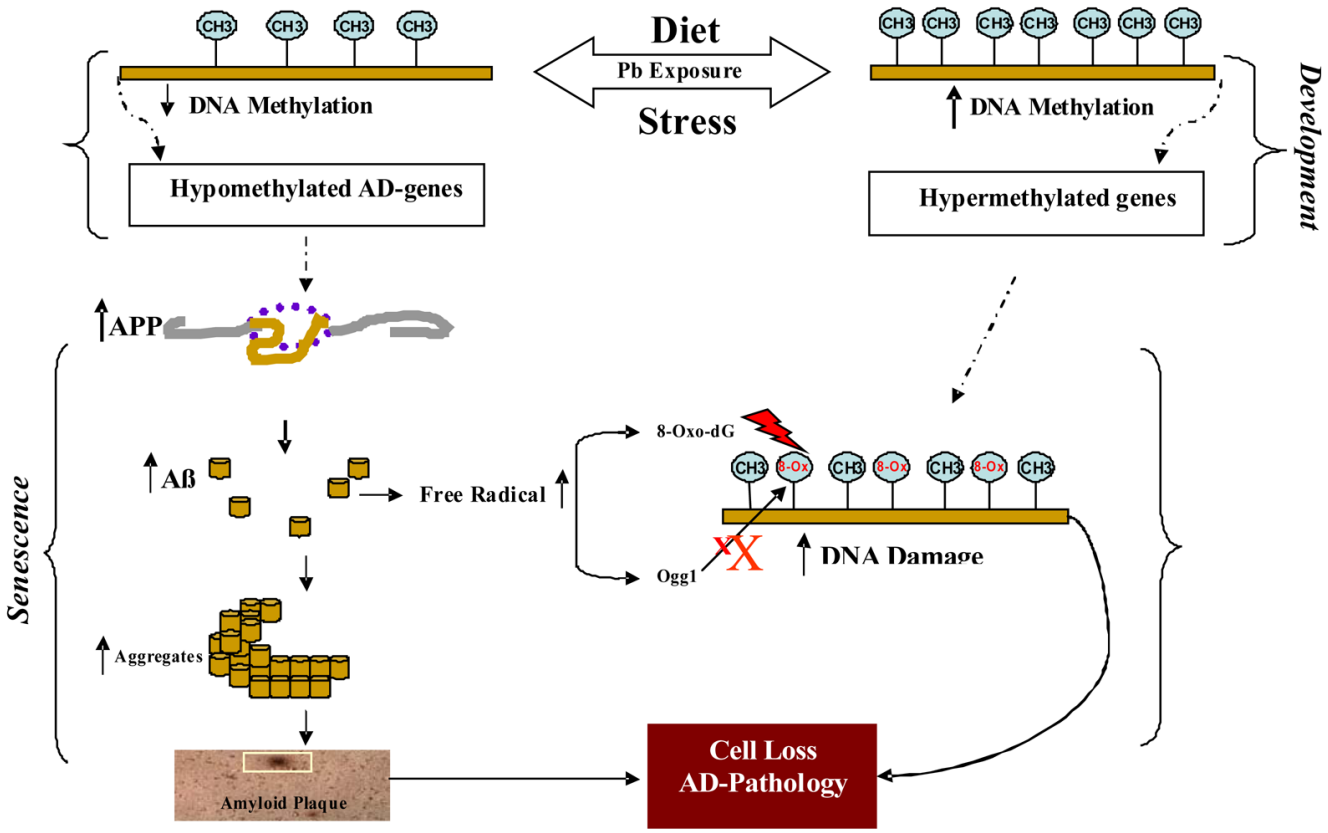

Figure 3. Epigenetic modifications during development and their impact on gene expression, DNA damage and neurodegeneration in the aging brain

It is presumed that DNA methylation during development sets the level of responsiveness of a gene for life. The higher the methylation burden, the more silenced a gene is. Exposure to Pb (or other perturbations) during development may inhibit DNA methylation of target genes such as APP. The inhibition of DNA methylation patterns resets the responsiveness of the APP promoter and the expression of the APP gene to a higher ceiling. The reset gene is over-expressed when challenged by an aging trigger. This leads to an increase in the production of APP and its amyloidogenic Aβ cleavage. Aβ forms aggregates and generates free radicals which attack macromolecules such as DNA. Exposure to Pb (or other perturbation) early in life may also enhance the methylation of some genes. The epigenetically modified genes may be more susceptible to oxidative stress later in life. Epigenetic modulations of 5-methylcytosine residues impair the capacity to repair adjacent oxidized guanine bases thereby rendering neurons more susceptible to damage. The increase in the levels of Aβ promotes aggregation, free radical formation, and DNA damage. These events enhance neurodegeneration and the formation of senile plaques in the aging brain.