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The Environment, Epigenetics and Amyloidogenesis

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Abstract Alzheimer's Disease (AD) is a progressive, irreversible neurodegenerative disease. Despite several genetic mutations (Haass et al., J. Biol. Chem. 269:17741–17748, 1994; Ancolio et al., Proc. Natl. Acad. Sci. USA 96:4119–4124, 1999; Munoz and Feldman, CMAJ 162:65–72, 2000; Gatz et al., Neurobiol. Aging 26:439–447, 2005) found in AD patients, more than 90% of AD cases are sporadic (Bertram and Tanzi, Hum. Mol. Genet. 13:R135–R141, 2004). Therefore, it is plausible that environmental exposure may be an etiologic factor in the pathogenesis of AD. The AD brain is characterized by extracellular beta-amyloid (Aβ) deposition and intracellular hyperphosphorylated tau protein. Our lab has demonstrated that developmental exposure of rodents to the heavy metal lead (Pb) increases APP (amyloid precursor protein) and Aβ production later in the aging brain (Basha et al., J. Neurosci. 25:823–829, 2005a). We also found elevations in the oxidative marker 8-oxo-dG in older animals that had been developmentally exposed to Pb (Bolin et al., FASEB J. 20:788–790, 2006) as well as promotion of amyloidogenic histopathology in primates. These findings indicate that early life experiences contribute to amyloidogenesis in old age perhaps through epigenetic pathways. Here we explore the role of epigenetics as the underlying mechanism that mediates this early exposure-latent pathogenesis with a special emphasis on alterations in the methylation profiles of CpG dinucleotides in the promoters of genes and their influence on both gene transcription and oxidative DNA damage.

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Introduction

AD is the most common form of dementia, currently affecting 20 million people worldwide (Goedart and Spillantini 2006). According to the United Nations population projection and other statistics, this number may cross 100 million in 50 years (Suh and Checler 2002; Liu et al. 2006). About 4.5 million cases are reported in the United States alone with an estimated 100,000 deaths every year among these patients (Hebert et al. 2003; Liu et al. 2006). AD is multi-factorial in origin; however, among all AD cases, only 3–5% is associated with familial or genetic factors. Mutations in certain genes viz., APP, Presenilin 1 (PS1), and Presenilin 2 (PS2) are implicated among the inheritable cases of AD (Cummings 2004). The genetics of AD have revealed that early onset AD (<60) is associated with APP or the presenilins (1 and 2), while susceptibility to late onset AD is linked to ApoE (Bertram and Tanzi 2004). Although such mutations may exert modest contributions to phenotypic consequences in the brain, they present in the population and may lead to a rise in Aβ levels, exacerbating the pathogenesis of AD. The genetics of AD play a role in a small percentage of patients, leaving the environment and other non-genetic factors responsible for the more common sporadic forms (Fig. 1).

The neuropathology of AD is characterized by senile plaques (SPs) and neurofibrillary tangles (NFTs). SPs are the extracellular deposits mainly composed of aggregated β-amyloid (Aβ) protein, whereas NFTs are formed due to the intracellular accumulation of hyperphosphorylated tau protein. The "amyloid cascade" hypothesis proposes that

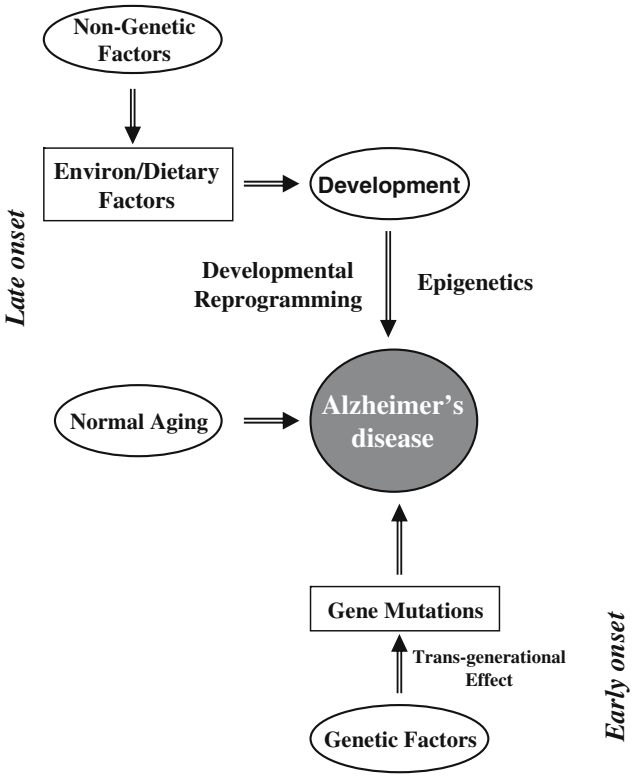

Figure 1 Potential contributors in the etiology of Alzheimer's disease. AD is a latent disease and aging is a risk factor associated with all forms of AD. While genetic or familial factors are involved in early-onset AD, more than 90% of AD cases are sporadic; devoid of genetic predisposition, and categorized under late-onset AD. The contribution of non-genetic factors such as environmental exposure and dietary factors has been proposed to play a crucial role in the etiology of sporadic AD perhaps through epigenetic pathways

abnormal processing of amyloid precursor protein (APP) results in excessive generation and accumulation of the amyloidogenic Aβ peptide (Hardy and Higgins 1992; Suh and Checler 2002). The most common sporadic forms of AD are associated with amyloidogenesis with abundant cerebral SPs in the brains of the patients (Spillantini et al. 1997; Selkoe 2001). Aβ is a cleavage product of the C-terminal of APP.

In addition to APP processing, de novo changes in APP levels can also influence Aβ production. Studies have provided clear evidence that any abnormality in the production of APP and its derivatives may cause detrimental damage to brain cells (Eckman et al. 1997; Feng et al. 2006). While many environmental insults such as oxidative damage and/or cellular energy depletion (Hoyer et al. 2005) can alter the expression levels of APP, our lab has demonstrated dynamic changes of APP expression in the 20 months old rat cortex long after neonatal exposure to Pb (Basha et al. 2005a; Bolin et al. 2006). The environmental metal Pb is known to produce detrimental effects to the central nervous system, and impact learning and cognition and is suspected to play a role in the onset of neurodegeneration (Cory-Slechta et al. 2007). Further studies using primates (Cynomolgus monkey) support the results of rodent studies. These findings form the basis for our hypothesis that environmental influences occurring during brain development may predetermine the expression and regulation of APP during old age, thereby altering the course of amyloidogenesis.

Developmental Disturbances, Pb Exposure and Amyloidogenesis

Events occurring early in life may have long lasting effects. Disturbances in growth and maturation during childhood have been associated with adult diseases such as stroke, hypertension and diabetes (Barker et al. 1989; Kramer and Joseph 1996; Emanuel 1997). Assessing such delayed consequences and compiling the epidemiological data on certain adult chronic diseases, Barker and his colleagues proposed the hypothesis called the "Fetal Basis of Adult Disease (FeBAD)". FeBAD suggests that injury at a critical period of organ development may cause programmatic changes, which consequently results in alterations in gene expression leading to functional deficits later in life. Since growth of the brain takes place during prenatal and infantile periods, it is also known that perturbations during this time may lead to cognitive decline during adulthood (Conel 1939; Tanner 1978; Jacobson and Jacobson 1991). Furthermore, several studies have previously suggested a link between early-life disturbances (risk factors) and the development of AD (Graves et al. 1996; Snowdon et al. 1996; Abbott et al. 1998).

Twin studies often used to confirm the inheritance pattern of a disease have shown poor concordance in neurodegenerative diseases such as AD and Parkinson's Disease (PD) (Gatz et al. 1997, 2005; Raiha et al. 1997). The negative findings of such studies along with the sporadic nature of late-onset AD suggest a strong role for the environment. In 1999, a population-based case-control study found that chronic occupational exposure to Pb as well as other metals was associated with Parkinson's disease (Gorell et al. 1999). In 2002, Kamel et al., evaluated the relationship between Pb exposure and Amyotrophic Lateral Sclerosis (ALS) (Kamel et al. 2002). They found that the risk for ALS was associated with elevations in both blood and bone Pb levels suggesting that Pb exposure played a role in the etiology of ALS. While these studies provided hints as to the possible connection between Pb exposure and neurodegenerative disease, more convincing evidence was provided by Stewart et al., in 2002. They analyzed occupational exposure to Pb and risk factors for AD. In this seminal work, Stewart et al., looked at tibia bone Pb levels in 529 former organo-lead workers and its

relationship to the ApoE genotype, a known risk factor for AD. They concluded that the persistent CNS effects of Pb are more toxic in individuals with at least one ApoE ε4 allele (Stewart et al. 2002). The link between past adult Pb exposure and neurodegeneration was further established by this group using brain MRI imaging (Stewart et al. 2006) and was consistent with their previous work which showed an association between Pb exposure and longitudinal cognitive decline.

The possibility that toxic levels of Pb in any form could result in the formation of Alzheimer's neurofibrillary tangles was studied from the findings on a patient who survived from severe Pb encephalopathy at 2 years of age, but died of severe mental deterioration at the age of 42 (Niklowitz and Mandybur 1975). The brain revealed that numerous pyramidal cells of the forebrain grisea contained Alzheimer's neurofibrillary tangles while the remaining pyramidal cells of the hippocampi showed granulo-vacuolar degeneration. Also, many senile plaques were observed, predominantly in the atrophic temporal cortex. Atomic absorption spectrophotometry disclosed a tenfold increase of Pb in frontal and temporal cortices as compared to the control. A high concentration of Pb has also been reported in patients with diffuse neurofibrillary tangles with calcification (DNTC) (Haraguchi et al. 2001). Pb is thus suspected to play a role in the pathogenesis of DNTC, a form of presenile dementia.

We have reported that developmental exposure of rats to the xenobiotic metal Pb resulted in an early transient and a delayed over-expression (20 months later) of APP and its amyloidogenic Aβ product (Basha et al. 2005a). We also found elevations in the oxidative DNA marker 8-hydroxyguanine (8-oxo-dG) in older rats that had been developmentally exposed to Pb (Bolin et al. 2006). These findings suggested that environmental influences occurring during brain development pre-determine the expression and regulation of APP later in life, potentially influencing the course of amyloidogenesis and oxidative damage.

To examine whether these responses are species-specific and study these phenomena in a species closely related to humans, we acquired the brains of monkeys that had been similarly exposed to Pb as infants in the 1980s. Primates express amyloid plaques and other pathological features absent in wild-type rodents. Preliminary 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 staining for Aβ showed that early exposure to Pb altered the distribution of intracellular Aβ staining and plaque formation. Furthermore, we found that the activity of the selective DNA methyltransferase as well as the methylation pattern of the APP promoter were significantly decreased in these old monkeys, suggesting that alterations in epigenetic control of gene expression had occurred in the exposed animals (Basha et al. 2005b).

DNA Methylation and Amyloidogenesis

The epigenetic mechanisms that reprogram gene expression and promote the accumulation of oxidative DNA damage described above may be mediated through alterations in the methylation profile of CpG dinucleotides in gene promoters. The cytosine in such dinucleotides is the preferred base for DNA methylation, while the guanine is the site of oxidative damage. Alterations in the DNA methylation pattern can influence both gene transcription and oxidative DNA damage and thus impact amyloidogenesis.

Epigenetics is the study of inheritable changes in gene function that occurs without a change of DNA sequence. Epigenetic regulation is crucial in the development of organisms, since it is required to achieve either stable expression or repression of genes at various stages of development. DNA methylation is the most studied epigenetic modification associated with stable gene expression; however, histone deacetylation and chromatin remodeling are also among the covalent epigenetic modifications involved in the expression and inactivation of genes (Reviewed by Feil 2006).

DNA methylation is accomplished through specific enzymes called DNA methyltransferases, which transfer a methyl group to the cytosine of CpG dinucleotides. The cytosine DNA-methyltransferase (DNMT) genes play a critical role in the establishment of the transcriptionally repressive complex. The presence of 5-methylcytosine in the promoters of specific genes sequesters the binding of transcriptional factors and other proteins to DNA. It also recruits methyl-DNA-binding proteins and histone deacetylases to further block gene transcription sites. Therefore, DNA methylation is an important factor in gene silencing and the degree of promoter CG methylation plays a crucial role in regulating gene expression. Furthermore, DNA methylation and histone modifications are two molecular mechanisms that are linked and intertwined in epigenetic modulation of gene expression.

The APP gene promoter is abundant in CpG dinucleotides, which imparts the possibility of epigenetic regulation (Salbaum et al. 1989; Pollwein et al. 1992; Lukiw et al. 1994; Hoffman and Chernak 1995; Querfurth et al. 1999). Therefore, factors that perturb DNA methylation can alter APP expression and thus impact amyloidogenesis. For example, researchers have found increased plasma homocysteine (HCY) and decreased S-adenosylmethionine (SAM) levels in AD patients (Mulder et al. 2005; Obeid and Herrmann 2006). SAM is required by methyltransferase as methyl group donor and is demethylated into

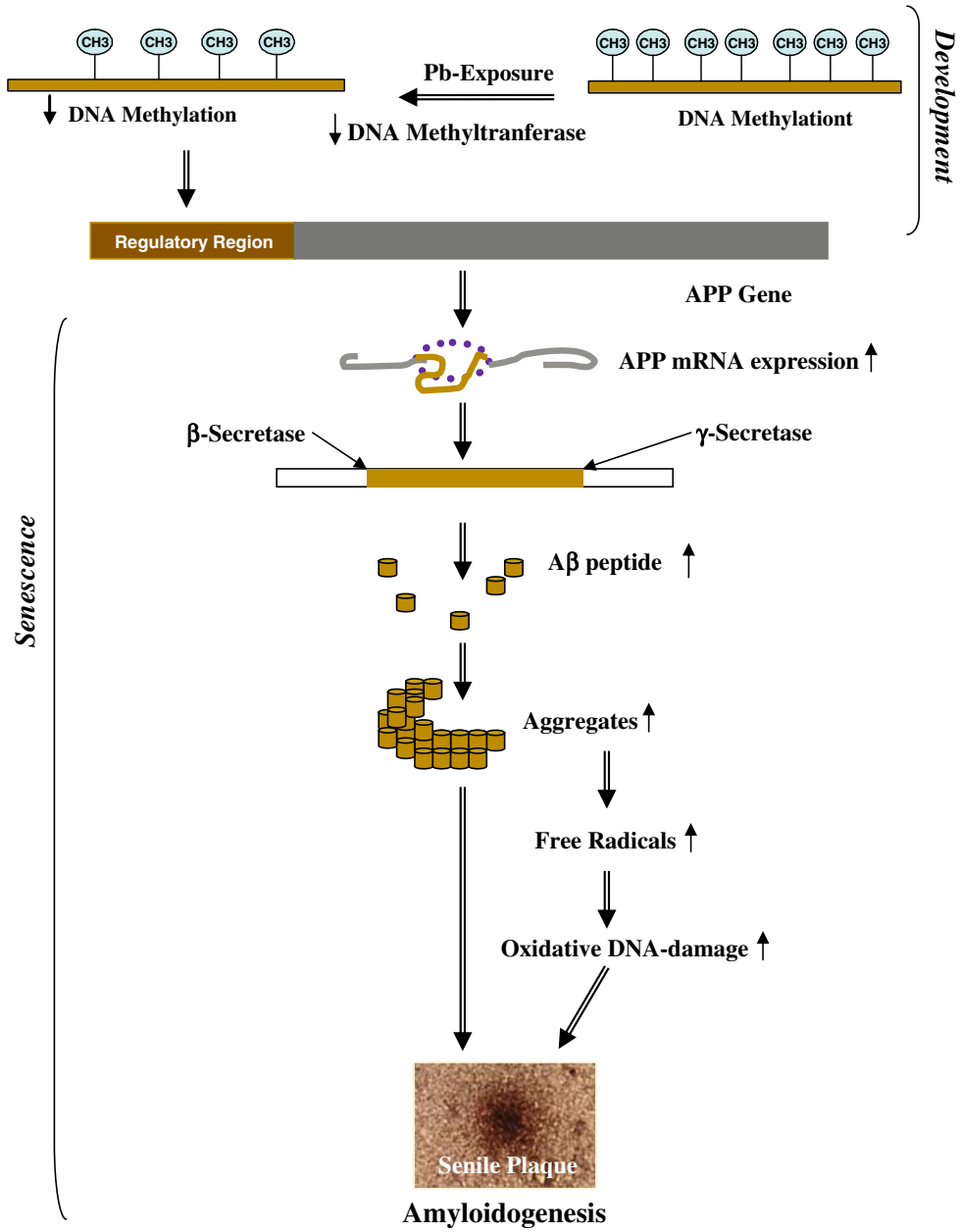

Figure 2 Developmental exposure to Pb and Amyloidogenesis during senescence. It is known that DNA methylation during development sets the level of responsiveness of a gene for life. The higher the methylation burden, the more silenced a gene is. Exposure to Pb during development may inhibit DNA methylation of target genes such as APP. The inhibition of DNA methylation patterns boosts the responsiveness of the APP promoter and the expression of the APP gene. This over expression of the APP gene increases the production of APP and its amyloidogenic Aβ cleavage product during senescence. Aβ forms aggregates and generates free radicals which attack macromolecules such as DNA. Epigenetic modulations of 5-methyl-cytosine 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

S-adenosylhomocysteine (SAH; an inhibitor of methyl-transferase), which hydrolyzes to produce HCY. This reaction is reversible which means increased HCY would lead to SAH accumulation and would cause DNA hypo-methylation. Furthermore, mice reared on a diet deficient in choline, essential for the biosynthesis of SAM, exhibit altered global and gene-specific DNA methylation patterns and APP is one of the genes showing over-expression with choline deficiency (Niculescu et al. 2005, 2006).

The relationship between the APP promoter methylation and APP gene expression has been explored. Rogaev et al. (1994) found those 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. More recently, Tohgi et al. (1999) and Nagane et al. (2000) found at least 13 potential methylation sites in the region −236 to −101 of the human APP promoter. These investigators suggested that age-related demethylation of cytosines have some significance in Aβ deposition in the aged brain (Tohgi et al. 1999). Furthermore, PS1 related to γ-secretase is regulated by its promoter methylation: administration or depletion of methylating factors respectively inhibits or promotes Aβ production (Clarke et al. 1998; Scarpa et al. 2003, 2006; Fuso et al. 2005).

DNA Oxidation and Amyloidogenesis

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 100–500 8-hydroxyguanosines (8-oxo-dG) daily in a human cell. Oxidative DNA damage is primarily repaired by the base excision pathway and base excision repair is initiated by a DNA glycosylase that recognizes a modified base. Oxoguanosine DNA glycosylase 1 (OGG1) is the major repair enzyme to remove 8-oxo-dG. Oxidative DNA damage is considered to be a central factor in the process of aging and aging-related diseases such as Alzheimer's disease.

Our study has shown that developmental Pb exposure increases Aβ levels as well as 8-oxo-dG production in old age (Bolin et al. 2006). Aβ is known to induce functional disturbances in vivo through its pro-oxidant and neurotoxic properties (Ono et al. 2006; Castellani et al. 2006). Aβ promotes the formation of reactive oxygen species (ROS) and the use of antioxidant can prevent Aβ elicited neurotoxic cascades (Mattson 1997; Yatin et al. 2000; Butterfield 2002; Obregon et al. 2006). Furthermore, the accumulation of HCY leads to increased cellular oxidative stress in which mitochondrial thioredoxin, and peroxiredoxin are decreased and NADH oxidase activity is increased (Tyagi et al. 2005).

Few studies have addressed DNA methylation and DNA oxidative damage simultaneously as an epigenetic phenomenon and little is known 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 (Valinluck et al. 2004). 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 (Weitzman et al. 1994; Turk et al. 1995). Methylated CpG has also been found to account for decreased transcription factor binding to the promoter region (Clark et al. 1997; Zhu et al. 2003).

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 that 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 and the levels of its gene products and their derivatives associated with amyloid formation.

Conclusion

We propose that environmental influences occurring during brain development alter the methylation pattern of the APP promoter which results in a latent increase in APP and Aβ levels. Increased Aβ levels promote the production of ROS which damage DNA. Epigenetic changes in DNA methylation impact both gene transcription and the ability to repair damaged DNA and thus imprint susceptibility to DNA damage. This susceptibility plus the programmed increase in Aβ levels via a transcriptional pathway programmed by environmental exposures in early life exacerbates the normal process of amyloidogenesis in the aging brain, thus accelerating the onset of AD. We have depicted this hypothesis using developmental Pb exposure as a model in Fig. 2.

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