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Novel Etiological and Therapeutic Strategies for Neurodiseases: Epigenetic Understanding of Gene–Environment Interactions

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Abstract. Epigenetics is a mechanism that regulates gene expression not depending on the underlying DNA sequence, but on the chemical modifications of DNA and histone proteins. Defects in the factors involved in epigenetic regulation cause congenital neurodevelopmental diseases, and thus, epigenetic regulation is essential for normal brain development. Besides these *intrinsic* defects, it is becoming increasingly apparent that *extrinsic* factors, such as insufficient nutrition, psychiatric drugs, and mental stress, also alter epigenetic regulation. Therefore, environmental factors may lead to "acquired" neurodevelopmental disorders through the failure of epigenetic regulation. Epigenetics is a biological key to understand the gene–environment interactions in neurodevelopmental disorders. As the mechanism is reversible, its comprehensive understanding will result in the development of new therapies for these disorders.

Keywords: epigenetics, DNA methylation, brain, environment, neurodevelopmental disorder, neurodisease

1. Introduction

The Ministry of Health, Welfare, and Labor in Japan has recently reported that the number of children with mild neurodevelopmental disorders, such as autism and pervasive neurodevelopmental disorders, is increasing by 10,000 cases per year (1). Similar trends are found in other countries, including the USA (2 – 4), in which the increase is partly attributed to social factors, such as "diagnostic substitution", in which children formerly diagnosed with mental retardation or learning disabilities are now diagnosed with autism. However, the increase in cases cannot be fully attributed to diagnostic substitutions, and it is possible that biological changes in the brains of the children are also involved in this increase.

Thanks to advances in genomic research, several genetic factors for autism have been identified. Mutations in genes encoding synaptic molecules have been identified in a subset of autistic children (5, 6). However, the increase in autism cannot be solely attributed to genetic factors because it is unlikely that mutation rates suddenly increased in recent years. Therefore, environmental factors are more likely to be involved in this increase.

The brain is sensitive to the dosage of gene products because either a deficiency or an overabundance of a neuronal molecule causes a similar abnormal brain condition. Deletion, mutation, or duplication of the *PLP1* gene causes the neurodevelopmental disorder Pelizaeus-Merzbacher disease (7); mutation or duplication of *PMP22* causes the adult-onset neuromuscular disease Charcot-Marie-Tooth disease (8); and it was recently demonstrated that not only a deficiency (9) but also an overabundance of the neuronal migration factor LIS1 causes severe mental retardation (10). These clinical findings suggest that the brain is extremely sensitive to perturbations in gene-regulation.

Epigenetics refers to a heritable phenotype coded by DNA methylation and histone modifications, which determines a unique gene expression profile in each cell. Epigenetic modification patterns are faithfully preserved after cell division, and this stability is essential for the maintenance of each distinct cell type throughout the life of an individual. However, epigenetic modifications are potentially changeable, because the addition or removal

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of each modification mark is mediated by specific enzymes. Furthermore, it is becoming increasingly apparent that the modifications are more vulnerable to environmental factors than the underlying sequence (11). This may be partly because there is no *intrinsic* repair system for epigenetic errors. At least, a comparable system as that seen for nucleotide sequence errors has not been identified. Therefore, the maintenance of a normal epigenetic pattern is important to perpetuate proper brain function throughout life.

In this article, we reviewed 1) epigenetic errors in congenital neurodevelopmental disorders, 2) environmental factors that could affect epigenetic mechanisms and are potentially associated with "acquired" (neurological symptoms start after birth) neurodevelopmental disorders, 3) medical interventions based on epigenetic reversal, and we discuss 4) the future goals of epigenetic therapies.

2. Epigenetics in congenital neurodevelopmental disorders

Epigenetic gene regulation is an essential mechanism for normal brain development (12) and defects in the molecules associated with this mechanism are known to cause congenital neurodevelopmental disorders (13 – 21). Genomic imprinting was the first epigenetic phenomenon that was linked to the development of human diseases. Of the two parental alleles of an imprinted gene, one allele is active and the other allele is epigenetically inactive. Thus, the chromosomal deletion of the active allele of an imprinted gene leads to no expression, resulting in a neurodevelopmental disorder, such as Prader-Willi syndrome (13).

As the X chromosome has more genes than the Y chromosome, females (XX) have more genes than males (XY). To compensate for this sex discrimination, one of the two female X chromosomes is epigenetically inactivated (14). Recent studies in cloned animals produced by somatic nuclear transfer have demonstrated that failure of X-chromosome inactivation induces embryonic abortion (22, 23) and suggest that X inactivation is essential for females to survive. When one of the X chromosomes is extremely small due to a large deletion, females with two active X chromosomes can avoid abortion and barely survive. However, these females show a severe neurodevelopmental delay (15).

DNA methylation refers to the addition of a methyl group (CH_3) to cytosine residues followed by a guanine (CpG) by DNA methyltransferases (DNMTs). Mutations in DNMT3B cause a congenital immunodeficiency disease, ICF syndrome, which is characterized by the three major features of immunodeficiency, centromere instability, and facial abnormalities, with mild mental retardation (16 – 18).

Methyl-CpG binding proteins (MBDs) are also essential proteins for epigenetic regulation and they bind to target genes and suppress their expression. Mutations in one of the MBDs, methyl CpG binding protein 2 (MeCP2), cause the autistic disease Rett syndrome (19, 20), in which dysregulation of the target neuronal genes occurs in the brain. These targets were recently identified as MeCP2, brain derived neurotrophic factor (BDNF), distal-less homeobox 5 (DLX5), and insulin-like growth factor binding protein 3 (IFGBP3) (24 – 26). Based on the current genetic understanding that autism is caused by congenital defects (i.e., mutations) in synaptic molecules (5), it is becoming apparent that epigenetic dysregulation of these molecules may also be attributed to autism (27 – 29). Our recent findings suggest that MeCP2 deficiency causes dysregulation of a certain synaptic scaffolding protein and neuronal cell adhesion molecules (T. Hirasawa and K. Miyake, unpublished data).

3. Environmental factors and epigenetics in acquired neurodevelopmental disorders

Environmental factors (e.g., toxins and infections) and genetic factors (e.g., mutations in synaptic molecules) have been discussed with respect to autism (5, 6). However, the biological mechanism that links these two groups has not been identified. Epigenetics may bridge the two groups (11) (Fig. 1). Besides the *intrinsic* or congenital epigenetic defects described above, several lines of evidence suggest that *extrinsic* or environmental factors, such as malnutrition, drugs, mental stress, maternal care, and neuronal stimulation, alter the epigenetic status, thereby affecting brain function (29 – 34). Therefore, it is intriguing to think that acquired neurodevelop-

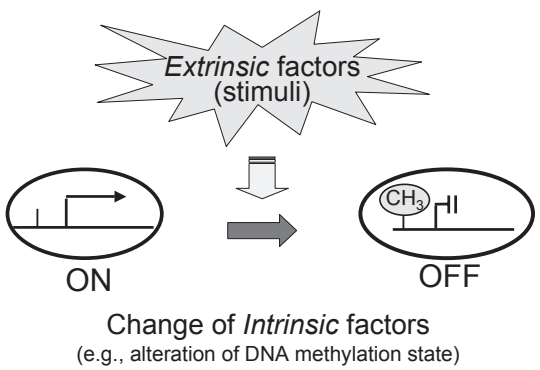

Fig. 1. Schematic illustration of the relationship between *extrinsic* and *intrinsic* epigenetic factors. *Extrinsic* environmental factors stimulate *intrinsic* epigenetic factors to change gene expression.

mental disorders (with an onset after birth) may be the result of epigenetic dysregulation caused by environmental factors (Fig. 2).

An example of an *extrinsic* factor is imipramine, a major antidepressant. In the depressive state, BDNF is downregulated in the hippocampus, and chronic treatment with antidepressants prevents this downregulation (35, 36). Recent studies have shown that the downregulation of BDNF is caused by increased repressive histone methylation of the *Bdnf* promoters, and imipramine reverses this downregulation by increasing histone acetylation (33). A major anticonvulsant, valproic acid (VPA), is another fascinating reagent that alters the epigenetic state of the brain. VPA blocks seizure-induced aberrant neurogenesis and protects animals from seizure-induced cognitive impairment; this is achieved by normalizing histone acetylation-dependent gene expression within the hippocampus, since VPA is a histone deacetylase (HDAC) inhibitor (31).

Epigenetics is also involved in the biological mechanisms of drug addiction. The persistence of drug addiction to cocaine and alcohol is indicated by changes in gene expression in the mesolimbic dopaminergic and glutamatergic systems, and these changes to the stable expression of genes in neurons are mediated by epigenetic mechanisms that alter the chromatin structure on specific gene promoters (37, 38). These findings suggest that chromatin-modifying enzymes, such as HDAC or DNMT, might be useful targets for the development of new psychiatric treatments (39).

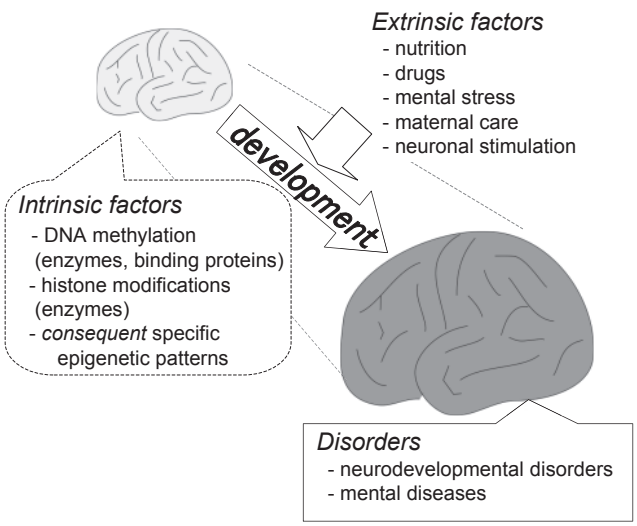

Fig. 2. Schematic illustration of the examples of *extrinsic* and *intrinsic* epigenetic factors. According to the concept, *extrinsic* environmental factors affect *intrinsic* epigenetic factors. These potentially cause neurodevelopmental disorders and mental diseases.

4. Epigenetic medical intervention

Nutritional factors, including folic acid, *S*-adenosylmethionine (SAM), and vitamins B6 and B12, are essential substrates for DNA methylation. Excess supplementation of folic acid in pregnant mice induces hypermethylation of genes in the fetuses (40). On the contrary, folic acid deficiency induces aberrant expression of normally methylated imprinted genes (41).

Methylation substrates have been used for the treatment of autistic and depressed patients for more than 30 years (42 – 45). Folic acid is now known to be effective for autistic patients with low levels of folic acid in their cerebral fluid but without *MECP2* mutations (46), suggesting that the MeCP2 targets may be hypomethylated in these patients, which leads to aberrant expression in the brain causing Rett-like clinical features. Administration of methylation substrates might ameliorate the methylation status of autism-related genes. We identified one MeCP2 target that showed an increase of DNA methylation with administration of SAM in cultured neuronal cells (M. Soutome, unpublished data). These findings suggest that the sufficient supply of nutrients related to methyl group metabolism is essential for the maintenance of normal brain function.

A study using the latest gene-manipulation technology in mice has shown that Rett syndrome is treatable even after birth. *Mecp2*-knockout mice mimic the neurological symptoms observed in Rett syndrome patients, such as seizures, ataxic gait, and hind-limb clasping (12). A new mouse model has been created based on this first-generation *Mecp2*-knockout mouse, in which the inserted endogenous gene is artificially silenced and can be conditionally activated under the control of regulatory elements (47). After the development of neurological symptoms in the mice, the *Mecp2* gene was reactivated by the injection of the estrogen analog tamoxifen that targets the regulatory elements. As a result, the symptoms of the mice became milder and they survived much longer than the standard *Mecp2*-knockout mice. These results indicate that the developmental absence of MeCP2 does not irreversibly damage neurons and the subsequent neurological defects are not irrevocable, and they further suggest that neurodevelopmental disorders caused by epigenetic dysregulation could potentially be treatable after birth.

As mentioned above, the proper intake of nutrients, such as folic acid in green vegetables for DNA methylation and sulforaphane in cruciferous vegetables (broccoli) for histone acetylation, is essential to maintain a normal epigenetic status (48). However, when we think of the treatment of epigenomic diseases, in which the epigenetic state of specific genomic regions is aberrant, we need to

have a methodology that allows us to target gene-specific epigenetic recovery. One such candidate may be the use of small double-stranded RNA molecules designed within the target region of a specific gene promoter region (49, 50).

5. Future perspectives in epigenetic medicine

It has recently been reported that the epigenetic state of monozygotic twins becomes different with aging (51). This clearly suggests that epigenetics is affected by environmental factors in humans. Thus, one of the best approaches to identify metastable epigenetic regions is to search for such regions in monozygotic twins, where the genetic differences would be negligible. An epigenetic study in disconcordant twins, in which one of the twins has neurological symptoms, may help to identify the symptom-related epigenomic regions.

Studies using cultured neuronal cells and animal models are also important for the identification of environmental factors that affect *intrinsic* epigenetics. Such factors may include plastic materials, drugs for mental diseases, and multiple nutrients. Since in vitro fertilization (IVF) potentially increases the risk of an imprinted disease, Angelman syndrome (52, 53), the medium that is used in IVF may induce epigenetic changes in the fertilized eggs. In studies designed to identify specific epigenomic regions under certain environmental conditions, new epigenomic scanning methods and platform facilities to screen for abnormal behavior and emotionality in mice will be useful (54, 55).

So far, transgenic or knockout mice have been created and used to elucidate the pathogenesis of diseases. For next-generation mouse research, mice will be used for the assessment of environmental deteriorating or ameliorating factors for neurological diseases. *Mecp2*-knockout mice have already been used for this purpose (56, 57); and autism mouse models either with gene-knockout, chromosomal duplication, or *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis will also be good candidates to search for environmental factors that affect brain development (58; 59; S. Wakana et al., submitted). Identification of epigenomic alterations in these mice will identify treatable genomic targets.

To elucidate the pathogenesis of neurodevelopmental disorders with defects of genetic factors related to epigenetic mechanisms such as imprinted diseases and Rett syndrome (45), numerous studies have been performed using postmortem brain samples of patients and brain samples of knockout model mice. However, postmortem brain samples of patients may not maintain their original pathogenic nature because of secondary changes caused by their clinical symptoms (e.g., hypoxic changes by recurrent seizures) and observations in brain samples from knockout model mice are potentially different from those observed in the patients' brains. In this context, findings from cultured neuronal cells differentiated from induced pluripotent stem (iPS) cells derived from patients (60) may shed light on the consequence of epigenetic failures in neurons (e.g., which type of neurons, dopaminergic or serotonergic, are predominantly affected by defects in an epigenetic molecule (e.g., MeCP2). Since genetic differences in copy number variation (CNV) between identical twins have recently been reported (61), it may be intriguing to investigate the genetic effects on neurons using differentiated iPS cells derived from disconcordant twins with a neurodevelopmental disorder.

It has been believed that epigenetic changes or errors are erased and reset through germline transmission. However, recent reports have shown that epigenetic alterations (epimutations) are potentially transmitted to the next generation (62, 63), although these findings are still controversial (64). It has recently been recognized that women who do not take a sufficient amount of essential nutrients, such as folic acid, during pregnancy are increasing in Japan, which increases the risk of having babies with neural tube defects (65). An inappropriate supply of nutrients from the mother to the fetus also increases the susceptibility of the fetus to develop diabetes mellitus by changing their epigenetic status (66).

In summary, research to identify the environmental and social factors associated with neurodevelopmental diseases are required in Japan where the number of children with mild developmental delays is increasing, and epigenetics may be an ideal solution for this purpose, partly because epigenetic alterations, identified in children, can be reversible and thus treatable.

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