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REVIEW

DNA methylation of the *BDNF* gene and its relevance to psychiatric disorders

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Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor, which is important for neuronal survival, development and synaptic plasticity. Accumulating evidence suggests that epigenetic modifications of *BDNF* are associated with the pathophysiology of psychiatric disorders, such as schizophrenia and mood disorders. Patients with psychiatric disorders generally show decreased neural BDNF levels, which are often associated with increased DNA methylation at the specific *BDNF* promoters. Importantly, observed DNA methylation changes are consistent across tissues including brain and peripheral blood, which suggests potential usefulness of these findings as a biomarker of psychiatric disorders. Here we review DNA methylation characteristics of *BDNF* promoters of cellular, animal and clinical samples and discuss future perspectives.
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INTRODUCTION

Neurotrophic factors are known to be important regulators of neural survival, development, function and plasticity in central and peripheral nervous system. To date, nerve growth factor, neurotrophin-3 (NT-3), NT-4/5 and brain-derived neurotrophic factor (BDNF) have been characterized as members of neurotrophic factors. Among them, BDNF was purified and cloned in 1982 as the second member. Subsequent studies revealed that BDNF is a unique activity-dependent NT widely expressed in the brain and has long-term effects on neuronal survival, development and synaptic plasticity. Further studies showed that BDNF may be involved in the pathophysiology of a wide range of psychiatric disorders, such as schizophrenia, major depression and bipolar disorder. Of note, accumulating evidence suggests that epigenetic modifications that include DNA methylation of *BDNF* promoters are clearly associated with the pathophysiology of psychiatric disorders. In this paper, we review the findings on DNA methylation of *BDNF* promoters in cellular, animal and clinical studies and discuss future perspectives.

REGULATION AND FUNCTION OF BDNF

Rodent and human *BDNF* has a characteristic gene structure, consisting of multiple untranslated exons ($\geqslant 8$) and a single coding exon[1–6] (Figure 1). The untranslated exons contain separate promoters upstream of each exon. The coding exon encodes pro-BDNF protein as well as 5′ and 3′ untranslated regions.[6] The 3′ untranslated region of the coding exon also contains two polyadenylation sites. Expression of *BDNF* gene is spatiotemporally regulated by these different promoters and polyadenylation sites, yielding approximately 34 alternative splicing transcripts.[7,8] In addition, the *BDNF-AS (BDNF antisense* RNA), which gives rise to non-coding RNA to form dsRNA duplexes with *BDNF* mRNA *in vivo*, may further make the *BDNF* regulation complex.[4,7] *BDNF* is widely expressed throughout the brain, including the frontal cortex, hippocampus, amygdala, corpus callosum, basal ganglia, thalamus, brainstem, pons and cerebellum.[7,9,10] Regardless of the type of splice variants, all mRNAs are translated into single pro-BDNF proteins. The pro-BDNF protein is then cleaved into a mature form of BDNF by different enzymes, including furin within the endoplasmic reticulum, proconvertases enzymes within secretory vesicles and tissue plasminogen activator/plasmin in the extracellular space.[11] The amino-acid sequence of the mature human BDNF is identical to that of porcine, rat and mouse,[12,13] and is 90% identical to fish BDNF,[14] indicating that the *BDNF* gene is highly conserved during vertebrate evolution.

Mature BDNF protein is secreted as a basic protein that can bind to two distinct transmembrane receptors, p75 NT receptor ($p75^{NTR}$) and TrkB.[15,16] Binding of BDNF to TrkB leads to phosphorylation of tyrosine residues in the subcellular domain of the TrkB and activates downstream signal cascades that are essential for neural differentiation and survival, neurite outgrowth and synaptic plasticity.[16] On the other hand, pro-BDNF preferentially binds to $p75^{NTR}$ and induces neuronal apoptosis and hippocampal long-term depression.[17–19]

The importance of BDNF in neural development and survival is confirmed by animal studies. Most of homozygous *Bdnf* mutant mice die within 2 days after birth, but some survive for 2–4 weeks. They show striking behavioral phenotypes of spinning, head bobbing and hindlimb extension, with deficiencies in coordination of movements

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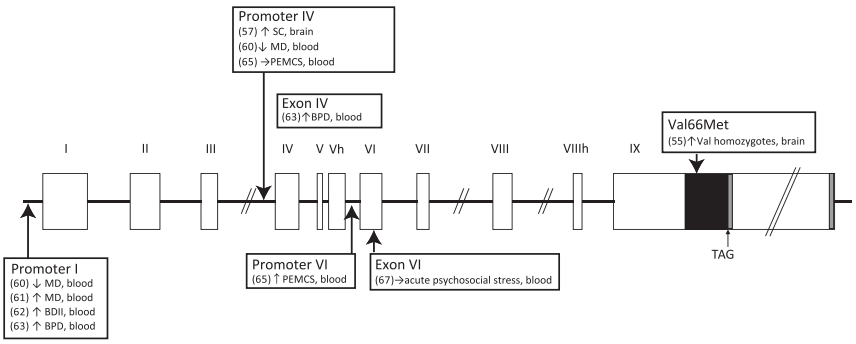

Figure 1 Structure and DNA methylation studies of the human *BDNF* gene. Exons are represented as boxes and the introns as lines. The roman numerals indicate the numbers of exons. The black box represents the pro-BDNF protein. Two gray boxes in exon IX are the polyadenylation sites. BDII, bipolar disorder II; BPD, borderline personality disorder; MD, major depression; PEMCS, prenatal exposure to maternal cigarette smoking; SC, suicide completers. Direction of DNA methylation changes are indicated by arrows.

and balance. Interestingly, structural abnormalities of central nervous system in *Bdnf* mutant mice are relatively mild, although *Bdnf* influences the development of the cortex, hippocampus, midbrain, cerebellum and structures in other brain regions.[20]

POSSIBLE ROLES OF *BDNF* IN MENTAL DISORDERS

The human *BDNF* gene is located on chromosome 11p13.[21,22] The Val66Met polymorphism (rs6265) in the *BDNF* gene is associated with the intracellular trafficking and processing of pro-BDNF protein.[23] A meta-analysis of case-control studies confirmed that this polymorphism had association with the risk of schizophrenia and other mental disorders, such as substance-related disorders and eating disorders.[24] This analysis demonstrated that individuals with the Met/Met homozygous allele showed a 19% higher risk of developing schizophrenia and other psychotic disorders than those with the Val/Met heterozygotes. Though initial magnetic resonance imaging studies showed that individuals with Met allele have smaller volumes of the frontal gray matter and hippocampus, and larger CSF volume than Val homozygotes,[25] it is cautioned that such finding might be subject to publication bias.[26]

Postmortem studies generally reveal the downregulation of *BDNF* expression in patients with schizophrenia and mood disorders. In schizophrenia, expression levels of *BDNF* in the prefrontal cortex, parietal cortex and hippocampus were decreased.[27–30] However, some early studies reported increased BDNF levels in the brains of patients with schizophrenia.[31–33] In patients with bipolar disorder, decreased BDNF levels are reported in the frontal cortex and hippocampal CA4 region, compared with controls.[34–36]

Using the peripheral blood, a large number of clinical studies have been conducted to examine the relationship between BDNF levels and diagnosis. In major depression, several meta-analysis indicated that serum BDNF level is significantly decreased in patients.[37–39] In schizophrenia, a significant reduction of serum BDNF level was also reported in both first-episode patients (patients who had experienced a first acute psychosis) and chronic-medicated patients (patients suffering from schizophrenia for a long term), compared with healthy controls.[40] Recent meta-analysis further demonstrated that serum BDNF level was reduced in both medicated and drug-free patients with schizophrenia regardless of medication dosage.[41]

DNA METHYLATION OF *BDNF*: CELLULAR AND ANIMAL MODELS

The importance of DNA methylation in the regulation of *Bdnf* expression was firstly identified in the experiments using cultured rat neurons.[42,43] Membrane depolarization artificially induced by potassium chloride led to a decrease of DNA methylation at promoter IV and an increase of transcription from this promoter. Subsequent studies demonstrated that membrane depolarization decreased expression levels of *Dnmt1* and *Dnmt3a* in cultured mouse cortical neurons and concomitantly decreased DNA methylation levels at the promoters I and IV.[44,45] Similarly, 5-aza-2-deoxycytidine, a DNA methyltransferase inhibitor, induced demethylation at *Bdnf* promoter I in mouse Neuro-2a cells. This demethylation was associated with upregulation of *Bdnf* gene expression.[46]

In animal models, infusion of zebularine, another DNA methyltransferase inhibitor, to the rat hippocampus CA1 region significantly decreased DNA methylation levels at promoters I, IV and VI. Expressions from these promoters are concomitantly increased.[47] In serotonin transporter knock-out rats, increased DNA methylation at promoter IV and downregulation of gene expression from this promoter has been reported.[48] A similar correlation has been observed in chicken. A study reported that the expression levels of two alternative splicing variants of chicken BDNF: cBDNF 1 and 2 were negatively regulated by the DNA methylation levels in their promoters.[49] These results suggested that the correlation between DNA methylation and gene expression of *BDNF* gene in the brain is well conserved during vertebrate evolution.

Various environmental stimuli can affect the epigenetic status of *Bdnf* gene.[50] For example, both Bdnf mRNA and protein levels in the visual cortex of adult rats, which were reared under light-deprivation condition, were significantly decreased, and they were associated with increased DNA methylation level at *Bdnf* promoter IV.[51] Male mice born from methylmerculy-exposed mother showed a significant decrease of *Bdnf* in the dentate gyrus with a significant increase in DNA methylation at *Bdnf* promoter IV.[52] Psychosocial stress such as early-life stress and post-traumatic stress also induced downregulation of exon IV mRNA and increase of DNA methylation in the prefrontal cortex and hippocampus.[53,54]

DNA METHYLATION OF *BDNF* IN THE POSTMORTEM BRAIN TISSUES OF PATIENTS WITH PSYCHIATRIC DISORDERS

DNA methylation studies of *BDNF* promoters using human brain and blood samples are summarized in Figure 1 and Table 1. In the prefrontal cortex, subjects with Val/Val homozygote for Val66Met polymorphism showed a higher DNA methylation level compared with Met carriers in the vicinity of SNP site (average methylation level was 83% for Val/Val and 78% for Met carriers).[55] Another study revealed that DNA methylation level of the promoter region of *BDNF*

Table 1 DNA methylation studis of BDNF using human samples

Author (ref.)	*Year*	*Sample*	*Tissue*	*Locus*	*Expression*	*Method*	*Findings*
Mill *et al.*[55]	2008	35 SZ, 35 BD, 35 CT	Postmortem brain (prefrontal cortex)	Coding exon around rs6265 (Val66Met polymorphism) and three promoter regions	ND	Pyrosequencing	Higher methylation in Val homozygotes than Met carriers No statistical differences at promoter regions
Keller *et al.*[57]	2010	44 SC, 33 CT	Postmortem brain (Wernicke area)	Promoter IV	ND	Pyrosequencing, bisulfite colony sequencing, MassArray	Increase of DNA methylation at specific CpG sites in promoter IV in SC
Toledo-Rodriguez *et al.*[65]	2010	26 PEMCS (without drugs), 52 PEMCS (with drugs), 38 non-PEMCS (without drugs), 40 non-PEMCS (with drugs)	Peripheral blood	Promoters IV, VI	ND	Bisulfite colony sequencing	Increase of DNA methylation of the promoter VI but not of the promoter IV in PEMCS. No significant association between the level of drug experimentation and DNA methylation
Fuchikami *et al.*[60]	2011	20 MD, 18 CT	Peripheral blood	Promoters I, IV	ND	MassArray	Complete separation of patients with MD from CT based on the methylation profiles of promoter I Most of the CpG sites in promoter I showed hypomethylation in MD
D' Addario *et al.*[62]	2012	49 BDI, 45 BDII, 52 CT	Peripheral blood	Promoter I	Downregulation of gene expression in BDII	Fluorescence-based methylation-specific real-time PCR	Increase of methylation in BDII Negative correlation between gene expression and DNA methylation in BDII Higher level of DNA methylation in patients on antidepressant compared with antidepressant-free patients Reduction of methylation level in subjects under therapy with mood stabilizers Reduction of methylation level in patients in mania/hypomania/mixed status compared with subjects in euthymia and/or in depression
Davies *et al.*[66]	2012	9 CT (elderly)	Peripheral blood, cortex, cerebellum	Around the intragenic CGI region of the BDNF gene	ND	MeDIP-seq, Pyrosequencing	Hypermethylation in blood compared with cortex and cerebellum from the same individuals
Rao *et al.*[35]	2012	10 AD, 10 BD, 10 CT	Postmortem brain (frontal cortex, BA9)	Promoter	Decrease of mRNA in the AD brain	Fluorescence-based real-time PCR	Increased DNA methylation in AD and BD
Kordi-Tamandani *et al.*[59]	2012	DNA: 80 SZ, 71 CT. RNA : 17 SZ, 17 CT	Peripheral blood	Promoter	Downregulation of gene expression in SZ	Methylation-specific PCR	Increased frequency of methylated alleles in SZ
Unternaehrer *et al.*[67]	2012	76 CT (Trier social stress test)	Peripheral blood	3' end of exon VI	ND	MassArray	No differences in methylation status between pre- and post-acute psychosocial stress
Kim *et al.*[64]	2013	286 patients with recent ischemic stroke	Peripheral blood	Promoter	ND	Pyrosequencing	Association of higher methylation status with worse outcomes at 1 year, and with worsening of physical disability and cognitive function No significant interactions between Val66Met genotype and DNA methylation
Keleshian *et al.*[56]	2013	9 CT(middle aged), 10 CT (aged)	Postmortem brain (frontal cortex, BA9)	Promoter	Decrease of protein in the aged sample compared with middle-age group. No change in mRNA expression	qPCR after digestion of methylation-sensitive restriction enzymes	Positive correlation between methylation level and age
D' Addario *et al.*[61]	2013	41 MD, 44 CT	Peripheral blood	Promoter I	Downregulation of gene expression in MD	Fluorescence-based methylation-specific real-time PCR	Increase of DNA methylation in MD Higher level of methylation in patients on antidepressant drugs alone compared with patients on antidepressants plus mood stabilizers No differences in methylation among mood status
Perroud *et al.*[63]	2013	115 BPD, 52 CT	Peripheral blood	Promoter I, exon IV	Higher protein levels in BPD than CT	High-resolution melt assay	Higher methylation status in BPD Higher methylation status in patients with greater number of childhood trauma Positive association between depression severity, hopelessness and impulsivity and methylation status

Abbreviations: AD, Alzheimer's disease; BD, bipolar disorder; BDI, bipolar disorder I; BDII, bipolar disorder II; BDNF, brain-derived neurotrophic factor; BPD, borderline personality disorder; CGI, CpG island; CT, control; MD, major depression; ND, not determined; PEMCS, prenatal exposure to maternal cigarette smoking; SC, suicide completers; SZ, schizophrenia.

was associated with age, and BDNF protein level was significantly decreased in the prefrontal cortex of elderly people.[56]

In psychiatric disorders, DNA methylation levels of *BDNF*-coding exon and three different promoter regions in the prefrontal cortex of patients with bipolar disorder or schizophrenia were examined by pyrosequencing.[55] However, both diseases did not show statistical DNA methylation differences compared with control subjects. On the other hand, one study reported significantly higher DNA methylation of *BDNF* in the prefrontal cortex of patients with bipolar disorder and those with Alzheimer's disease.[35] Significant increase of DNA methylation was also reported in the Wernicke area of suicide completers. At the promoter IV region, four CpG sites showed increased DNA methylation in suicide completers (average methylation level was about 5% for controls and 11% for suicide completers). Higher DNA methylation was generally associated with lower expression levels of *BDNF* mRNA in this study. The same research group also reported no alteration of the DNA methylation status of *TRKB* promoter.[57,58]

DNA METHYLATION OF *BDNF* IN THE PERIPHERAL BLOOD TISSUES OF PATIENTS WITH PSYCHIATRIC DISORDERS

In schizophrenia, one study assessed DNA methylation level of *BDNF* by methylation-specific PCR. Patients had more methylated alleles compared with control subjects and showed downregulation of *BDNF*.[59] In major depression, DNA methylation levels of *BDNF* promoters I and IV have extensively been examined. DNA methylation levels of 29 out of 35 CpG units in *BDNF* promoter I of patients were significantly different from those of controls.[60] Notably, most of the CpG units showed hypomethylation in depressed patients compared with those in controls in this study. By contrast, D'Addario *et al.*,[61] detected significant hypermethylation at *BDNF* promoter I in major depression by quantitative methylation-specific PCR procedure (about 32% for patients and 24% for controls). They further reported that DNA methylation level was significantly lower in patients treated with mood stabilizers. In bipolar disorder, the same group reported hypermethylation of the *BDNF* promoter I region in patients with bipolar disorder II, which is a form of bipolar disorder exhibiting a less intense manic episode (about 33% for patients and 24% for controls), but not in those with typical bipolar disorder (about 20%). Similarly to the previous report, patients treated with mood stabilizers showed lower DNA methylation level than those treated with other drugs.[62] Hypermethylations at promoters I and IV were also detected in patients with borderline personality disorder by using high-resolution melt analysis.[63] There was a significant positive association between depression severity, hopelessness, impulsivity and child trauma and *BDNF* methylation level. However, no association was found between BDNF protein levels and DNA methylation levels in this study. Another study regarding poststroke depression suggested that higher methylation level at *BDNF* promoter I was associated with incident poststroke depression and worsening of depressive symptoms over the 1-year follow-up.[64]

CONCLUSIONS AND PERSPECTIVES

Cellular and animal models clearly suggest that expression of *Bdnf* in neuronal cells is tightly regulated by DNA methylation of specific promoters. DNA methylation status of promoters I and IV was extensively studied, and they were found to be tightly regulated in the physiological condition. In response to environmental stimuli, methylation levels of these promoters can be actively increased or decreased and transcription from these promoters is altered accordingly. Whereas decreased DNA methylation level involves dissociation of MeCP2 from the promoter, the mechanism of increased DNA methylation at the specific sites remains largely unclear. In the human sample, aging and genotype affect the methylation status of *BDNF* promoters. In general, in the various psychiatric conditions, patients show decreased BDNF levels in the brain and in peripheral blood. In the examined cases, decreased BDNF levels were often associated with increased DNA methylation at the *BDNF* promoters. Very importantly, consistent DNA methylation difference was observed between brain and peripheral blood samples. Considering the advantage of peripheral samples over the postmortem brains in terms of accessibility and longitudinal traceability, in addition to pursuing the pathophysiology of psychiatric disorders, DNA methylation status of *BDNF* promoters in blood samples will be useful for developing biomarkers. Currently, increased DNA methylation of *BDNF* promoter has been identified in a wide range of psychiatric disorders. Defining the precise environmental factors that affect DNA methylation levels in patients will be needed. In addition, effect of the severity of disease, disease duration and effects of medication should also be thoroughly tested. Molecular mechanisms of altering DNA methylation levels at specific *BDNF* promoter sites will be further pursued using cellular and animal models.

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