Changes in BDNF expression and dendritic trafficking of select BDNF transcripts linked to epigenetic changes in BDNF Val66Met mice

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Epigenetic mechanisms, changes in chromatin condensation state that alter gene expression, have been shown to regulate brain function/dysfunction, including development, neurodegeneration, memory, drug addiction, and stress response. Several cellular mechanisms exist that may remodel the structure of chromatin activating transcriptional process for some genes and silencing for others, such as posttranscriptional modifications of histones and methylation of DNA. In particular, the acetylation on lysine residues of histone H3 is associated to activation of gene expression, while the trimethylation of H3K27 is repressive.

Brain Derived Neurotrophic Factor (BDNF), a key factor in neuroplasticity, gene expression, synaptic function and cognition, has been implicated in the pathophysiology of various neuropsychiatric and neurodegenerative disorders. In addition, the human Val66Met polymorphism in the BDNF gene that causes a Val/Met substitution in codon 66 of proBDNF was associated with major susceptibility to neuropsychiatric diseases. The BDNF Val66Met transgenic mouse is the only existing animal model that fully recapitulates the phenotypic hallmarks of the BDNF Val66Met human polymorphism. Indeed, both human and mice $BDNF_{Met}$ allele carriers show reduced hippocampal volume, cognitive deficits, increased anxiety-related behaviour and impaired extinction of fear conditioning, a type of learning involved in phobias and post-traumatic stress disorder (Chen et al., 2006; Soliman et al., 2010).

In order to analyze epigenetic changes induced in mice gene expression by the presence of the BDNF Val66Met polymorphism, chromatin immunoprecipitation (ChIP) of hippocampus (HPC) from wild-type ($BDNF^{Val/Val}$) and transgenic ($BDNF^{Met/Met}$) mice was performed with specific antibodies directed against DNA-bound histones, methylated or acetylated. Briefly, hippocampal chromatin was cross-linked to histone proteins, sonicated, and immunoprecipitated with antibodies anti-acetyl H3K9/14 (marker of gene expression activation), or anti-trimethyl H3K27 (marker of gene expression repression). ChIP was followed by quantitative real-time PCR (qPCR) analysis of immunoprecipitated DNA to determine levels of histone modifications at promoter all BDNF splice variants. The percent of input values from each qPCR data set were analyzed in two-tailed unpaired Student's t-test to determine statistical significance.

We have found that $BDNF^{Met/Met}$, compared with $BDNF^{Val/Val}$ mice, showed increased H3K27 trimethylation on BDNF-6 promoter in HPC, associated with reduced expression of BDNF-6, the main BDNF transcript that is activity-dependent translocated to dendrites and mediates the antidepressant action of drugs and physical activity (Chiaruttini et al., 2008; Baj et al., 2012). In addition, in vitro knockdown by RNA interference or inhibition by DZNep of the specific H3K27 histone methyltransferase Enhancer of Zeste Homolog 2 (EZH2) led to upregulation of BDNF-6, implying a role for EZH2 in the epigenetic mechanisms regulating BDNF-6 expression. By using in situ hybridization for BDNF-6 we found that translocation of BDNF-6 to distal dendrites of CA1 and CA3 HPC region is virtually abolished in $BDNF^{Met/Met}$ mice. We speculate that this selective change in $BDNF^{Met/Met}$ mice may be a reason for the reduced regulated secretion of BDNF at synapses (observed in $BDNF^{Met/Met}$ mice, Chen et al., 2006), and a distinct correlate of pathology in mice and men carrying the Met allele.

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Chen et al. (2006). Science. 314, 140-3.
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Soliman et al. (2010). Science. 327, 863-6.