0026-895X/07/7103-635–637$20.00
MOLECULAR PHARMACOLOGY
Vol. 71, No. 3
33266/3184073
Mol Pharmacol 71:635–637, 2007
Printed in U.S.A.

PERSPECTIVE

DNA (Cytosine-5) Methyltransferase Inhibitors: A Potential Therapeutic Agent for Schizophrenia

Jonathan M. Levenson

Department of Pharmacology and the Waisman Center, University of Wisconsin School of Medicine & Public Health, Madison, Wisconsin

Received December 6, 2006; accepted December 19, 2006

ABSTRACT

In this issue of *Molecular Pharmacology*, Kundakovic et al. (p. 644) present compelling evidence suggesting that the promoters for reelin and GAD67 are coordinately regulated. The regulation occurs at the level of DNA (cytosine-5) methylation. Moreover, the authors present evidence suggesting that pharmacologic inhibition of DNA methyltransferase results in reversal of methylation, loss of methyl-DNA binding proteins and relief of repression. Repression of both reelin and GAD67 has been implicated in the pathogenesis of schizophrenia. Therefore, these results suggest that the *reelin* and *GAD67* promoters are subject to continuous repression by DNA methyltransferase and that inhibitors of DNA methyltransferase represent a potential treatment for Schizophrenia.

Schizophrenia is a debilitating neurological disease that afflicts approximately 1% of Americans. The symptoms of schizophrenia are severe and include auditory hallucinations, generalized paranoia, and severe cognitive dysfunction. Because of these phenomena, patients with schizophrenia are unable to function in normal social situations and tend to be fearful, withdrawn, and exhibit disorganized thought and speech. Onset of schizophrenia occurs in late adolescence or early adulthood and is often associated with a precipitating stressful life event. Contemporary treatments relieve some symptoms; however, most persons with schizophrenia continue to experience symptoms throughout their lives.

The nature of schizophrenia suggests that the initial triggering event results in a robust set of neuroadaptations that significantly derange normal brain function. How then, could one event result in widespread maladaptive changes in neural function that last a lifetime? Given that the entire composition of the human brain turns over every 2 months, the triggering event must impinge upon a process that is stable or is perpetuated throughout the lifetime of an individual. DNA is perhaps the only molecular component of neurons that is not continuously degraded and resynthesized, and represents a unique and powerful substrate for storage of cellular information.

DNA exists in the nucleus as a highly compressed protein-DNA complex known as chromatin. In addition to compressing DNA into the nucleus, chromatin acts as a molecular platform for signal integration and long-term information storage (Levenson and Sweatt, 2005). For example, every cell in a metazoan must "remember" its phenotype. This information is stored in the cell in the form of stable marks applied directly to chromatin that result in permanent changes to chromatin structure, gene expression, and cellular physiology.

The process of marking DNA and its associated proteins is commonly referred to as epigenetics. Epigenetics has various meanings depending on the context that is used. The broadest definition of epigenetics refers to processes of transmission and perpetuation of information through mitosis or meiosis that do not rely on DNA sequence. In this context, epigenetics encompasses DNA, RNA, and protein-based mechanisms. As applied to the adult nervous system, epigenetics refers to a mechanism for stable maintenance of gene expression whereby the DNA or its associated proteins are physically marked. These marks come in a variety of forms

Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.106.033266.
Please see the related article on page 644.

ABBREVIATIONS: CpG, cytosine-guanine dinucleotide; DNMT, DNA (cytosine-5) methyltransferase; GAD67, glutamic acid decarboxylase 67.

(for review, see Levenson and Sweatt, 2005). Methylation of cytosine residues is an epigenetic mark applied directly to DNA. Methylation of a cytosine can occur only when it exists as a cytosine-guanine dinucleotide (CpG). Methylated CpG dinucleotides are bound by methyl-DNA binding proteins, which recruit chromatin-remodeling enzymes, such as histone deacetylases, and actively repress gene expression. DNA methylation is an enzymatic process governed by DNA methyltransferase and an as-yet-unidentified DNA methylase.

An increasing body of literature indicates that the adult nervous system uses epigenetic marks to chromatin for integration and storage of information. Moreover, there are several examples whereby a single, precipitating event in the lifetime of an organism results in an epigenetic mark to chromatin, lasting changes in expression of one or more genes, and lifetime changes in behavior (Weaver et al., 2004; Kumar et al., 2005; Champagne et al., 2006). Given the sudden onset and lifetime persistence of symptoms, it is reasonable to hypothesize that the pathogenesis of schizophrenia is due, at least in part, to aberrant epigenetic marking of one or more genes, resulting in lifetime changes in their expression.

Postmortem studies of gene expression in the brains from persons diagnosed with schizophrenia have revealed that expression of glutamic acid decarboxylase 67 (GAD67), an enzyme critical for synthesis of the inhibitory neurotransmitter GABA, is significantly reduced in interneurons. Moreover, expression of reelin, an extracellular matrix-associated protein important for development and cognitive function (Weeber et al., 2002; Qiu et al., 2006), is also significantly reduced in these same neurons. Animal models of reelin haploinsufficiency exhibit reduced GABAergic inhibitory tone and a deficit in sensorimotor gating, hallmarks of schizophrenia (Tueting et al., 1999; Qiu et al., 2006). As a whole, these results suggest that an aberrant decrease in *reelin* and *GAD67* gene expression could contribute to the pathogenesis of schizophrenia.

In this issue of *Molecular Pharmacology*, Kundakovic et al. (2007) explore the hypothesis that methylation of the proximal promoters of *reelin* and *GAD67* are coregulated and, if so, whether the methylation status could be manipulated using DNA (cytosine-5) methyltransferase (DNMT) inhibitors. Previous studies have demonstrated that a CpG island exists in the proximal promoter of reelin (Chen et al., 2002). CpG islands are regions in the genome in which CpG dinucleotides occur at a frequency greater than would normally be expected. It is noteworthy that although CpG islands in general are usually hypomethylated, postmortem studies suggest that the reelin promoter is actually hypermethylated in persons with schizophrenia (Chen et al., 2002; Abdolmaleky et al., 2005). This hypermethylation could explain the reduction in reelin expression associated with schizophrenia. Kundakovic et al. (2007) postulate that this hypermethylation could also serve as a basis for therapeutic intervention.

Kundakovic et al. (2007) exploited NT-2 neuronal progenitor cells to study the regulation of reelin expression. NT-2 cells are ideal for these studies because they exhibit low levels of reelin expression and hypermethylation of the reelin promoter (Chen et al., 2002), modeling some of the molecular derangements associated with schizophrenia. Long-term treatment of NT-2 cells with the DNMT inhibitor doxorubicin significantly increased expression of reelin and GAD67. It is surprising that the increase in reelin and GAD67 was preceded by a significant decrease in expression of DNMT1 protein. Further studies revealed that doxorubicin decreased levels of DNMT1 and the methyl-DNA binding protein MeCP2 and increased levels of histone acetylation at the reelin promoter. These results suggest that in NT-2 cells, at least, the reelin promoter is actively repressed through the action of DNMT, and this repression is mediated via MeCP2-dependent chromatin remodeling (Fig. 1A). Furthermore, these results support other findings suggesting that DNMT inhibitors may block DNMT activity by promoting degradation of DNMT (Fig. 1B), perhaps through formation of nonfunctional complexes (Yokochi and Robertson, 2004).

The findings of Kundakovic et al. (2007) are striking when one considers the ramifications for intervention in schizophrenia. Hypermethylation of the reelin and GAD67 promoters, whether due to a triggering event during development or during adolescence, represents an epigenetic

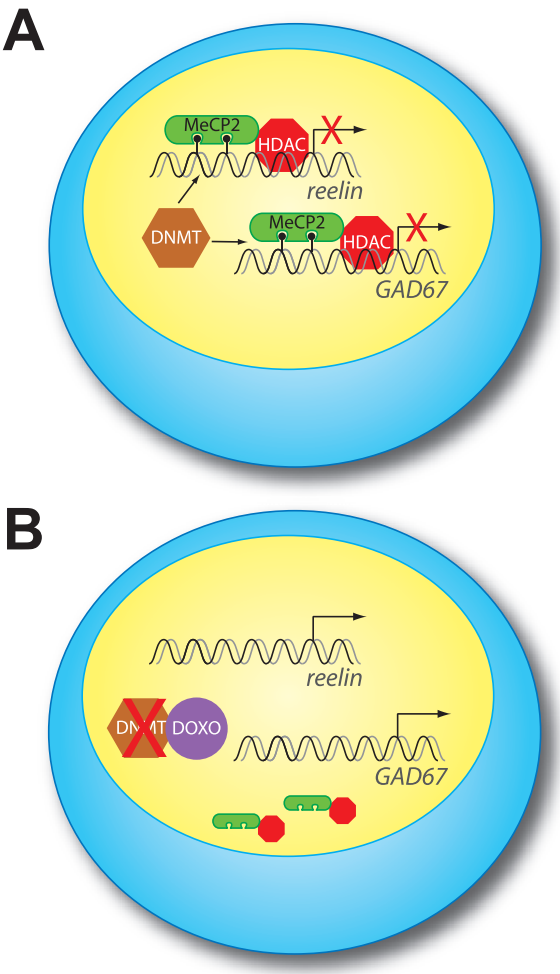

Fig. 1. Active repression of reelin and GAD67 by DNA methyltransferase. A, under normal conditions, the expression of reelin and GAD67 is repressed through active methylation of their promoters by DNMT. Methyl CpGs are bound by the methyl-DNA binding protein MeCP2, which recruits chromatin-remodeling enzymes including histone deacetylase (HDAC). This results in a decrease in histone acetylation and repression of gene expression. B, application of a DNMT inhibitor, such as doxorubicin (DOXO) results in formation of DNMT-DOXO complexes and degradation of DNMT. In the absence of DNMT, active methylation and repression of the reelin and GAD67 promoters is relieved, and expression of reelin and GAD67 can occur.

mark that persists for a person's lifetime. However, the use of DNMT inhibitors to treat schizophrenia or any disease is years away at best, because drugs of this nature will probably result in up-regulation of numerous genes that are actively repressed via DNMT. Despite the potential drawbacks, the ability to pharmacologically erase an epigenetic mark with DNMT inhibitors and reverse some or all of the derangements in brain function associated with schizophrenia is revolutionary.

References

Abdolmaleky HM, Cheng KH, Russo A, Smith CL, Faraone SV, Wilcox M, Shafa R, Glatt SJ, Nguyen G, Ponte JF, Thiagalingam S, and Tsuang MT (2005) Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. *Am J Med Genet B Neuropsychiatr Genet* **134:**60–66.

Champagne FA, Weaver IC, Diorio J, Dymov S, Szyf M, and Meaney MJ (2006) Maternal care associated with methylation of the estrogen receptor-alpha1b promoter and estrogen receptor-alpha expression in the medial preoptic area of female offspring. *Endocrinology* **147:**2909–2915.

Chen Y, Sharma RP, Costa RH, Costa E, and Grayson DR (2002) On the epigenetic regulation of the human reelin promoter. *Nucleic Acids Res* **30:**2930–2939.

Kumar A, Choi KH, Renthal W, Tsankova NM, Theobald DE, Truong HT, Russo SJ, Laplant Q, Sasaki TS, Whistler KN, et al. (2005) Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* **48:**303–314.

Kundakovic M, Chen Y, Costa E, and Grayson DR (2007) DNA methyltransferase inhibitors coordinately induce expression of the human *reelin* and *GAD67* genes. *Mol Pharmacol* **71:**644–653.

Levenson JM and Sweatt JD (2005) Epigenetic mechanisms in memory formation. *Nat Rev Neurosci* **6:**108–118.

Qiu S, Korwek KM, Pratt-Davis AR, Peters M, Bergman MY, and Weeber EJ (2006) Cognitive disruption and altered hippocampus synaptic function in Reelin haploinsufficient mice. *Neurobiol Learn Mem* **85:**228–242.

Tueting P, Costa E, Dwivedi Y, Guidotti A, Impagnatiello F, Manev R, and Pesold C (1999) The phenotypic characteristics of heterozygous reeler mouse. *Neuroreport* **10:**1329–1334.

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, and Meaney MJ (2004) Epigenetic programming by maternal behavior. *Nat Neurosci* **7:**847–854.

Weeber EJ, Beffert U, Jones C, Christian JM, Forster E, Sweatt JD, and Herz J (2002) Reelin and ApoE receptors cooperate to enhance hippocampal synaptic plasticity and learning. *J Biol Chem* **277:**39944–39952.

Yokochi T and Robertson KD (2004) Doxorubicin inhibits DNMT1, resulting in conditional apoptosis. *Mol Pharmacol* **66:**1415–1420.

Address correspondence to: Dr. Jonathan M. Levenson, Department of Pharmacology and The Waisman Center, University of Wisconsin School of Medicine and Public Health, 1300 University Avenue, Madison, WI 53706. E-mail: jlevenson@wisc.edu