COMMENTARY

Approaching the Molecular Pathology of Suicide

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Suicide risk and personality traits associated with suicidal behavior are often related to childhood maltreatment and other "environmental" stressors (1). Therefore, the pathophysiology of suicide is likely to involve molecular mechanisms that are not necessarily related to genetic factors (defined here as changes in DNA sequence), albeit this hypothesis is difficult to test conclusively.

Somatic cells (including neurons) employ a wide array of molecular mechanisms to alter and regulate gene expression and function without changing the sequence of the genomic DNA itself. These include (but are not limited to) "epigenetic" mechanisms, which are commonly defined by a chemical modification of the DNA and/or the histone proteins associated with it. For example, the methylation of DNA cytosine residues at the sites of CpG dinucleotides within gene promoters is an epigenetic mark typically associated with repression or downregulation of RNA transcription (2). In addition, gene expression is also regulated by a rich set of post-translational modifications of specific histone residues; these include lysine acetylation, methylation, SUMOylation and ubiquitinylation, arginine methylation, serine phosphorylation, and proline isomerization (2).

Now, two provocative articles, one by Poulter *et al.* in this journal (3) and the other one by McGowan *et al.* in PLoS ONE (4), provide evidence for DNA methylation changes in the frontal cortex (3) and hippocampus of suicide victims (4).

Poulter *et al.* (3) focused on the promoter of the ionotropic γ-aminobutyric acid *(GABA)-A a1* receptor subunit, which according to their previous work is expressed at decreased levels in the anterior prefrontal cortex of suicide victims (5). However, it should be noted that another study, conducted in anterior cingulate—which is in close proximity to the prefrontal cortex—reported a consistent increase in *GABA-A a1* expression in completed suicides (6). The clinical cohorts in both studies were defined by an underlying diagnosis of mood disorder, and therefore the discrepancy remains to be resolved. If not explained by demographic differences, medication history, postmortem variables, and other confounds, one interesting hypothesis would be that levels of *GABA-A a1* transcript show opposing alterations in different subdivisions of the frontal lobe of suicidal subjects. In any case, Poulter *et al.* (3) now report that a small subset of CpG dinucleotides at the proximal *GABA-A a1* promoter are hypermethylated in prefrontal cortex of 10 male suicide victims diagnosed with major depression, compared with non-psychiatric control subjects who died from other causes. The methylation levels in control subjects ranged from 6% to 10% but were as high as 38% in suicide victims. When taken together with their previous finding of decreased *GABA-A a1* messenger RNA (mRNA) expression in prefrontal cortex of suicide victims, the obvious implication is that an epigenetic mechanism, promoter DNA hypermethylation, underlies the observed deficit in *GABA-A a1* gene expression in depressed suicide victims. This is an interesting and also testable hypothesis. Poulter *et al.* (3) extracted DNA from prefrontal tissue homogenates and examined 18 CpG dinucleotides that reside within portions of the *GABA-A a1* promoter. There was suicide-related hypermethylation at 3 of 18 sites. Controlled studies ex vivo in cell culture or in vitro might be required to examine the impact of these site-specific methylation events on transcriptional activity. Also *GABA-A a1* transcript expression in the human prefrontal cortex shows laminar and cellular specificity, and therefore it will be important to clarify whether or not the suicide-related DNA methylation alterations (3) indeed reflect similar changes in the GABA-A a1 expressing neurons. Finally, to distinguish whether the observed *GABA-A a1* DNA hypermethylation is primarily related to suicide or, alternatively, to the underlying mood disorder or other psychiatric disease, it will be important to perform these studies with a psychiatric control group (depressed non-suicidal, for example).

Interestingly, the observed prefrontal *GABA-A a1* DNA hypermethylation in depressed suicide victims was accompanied by changes in DNA methyltransferase expression, including an increase in mRNA levels and immunoreactivity for DNMT3B (3). There was a negative correlation between *DNMT3B* and *GABA-A a1* expression both in the control and the suicide group, which indirectly suggests that DNMT-3B is the enzyme responsible for the hypermethylation of the GABA A receptor gene. Because DNMT3B expression is very low in differentiated tissues (including brain), the authors speculate that the observed upregulation in prefrontal cortex and other areas of suicide postmortem brain "might reflect a unique pathway that contributes to neuroplasticity" (3). Furthermore, in contrast to control subjects, at least some brain regions of suicide victims lacked a correlation between transcript levels of the three known DNA methyltransferases with catalytic activity (*DNMT3A, DNMT3B,* and *DNMT1*) (3). In light of these findings, which are based on quantitative reverse-transcriptase polymerase chain reaction extracts from tissue homogenates (3), it seems worthwhile to conduct detailed studies on the developmental regulation and regional and cellular expression patterns of the *DNMT3A, DNMT3B, DNMT1* and of the non-catalytic regulatory unit *DNMT3L*, in normal human brain. Likewise, it seems worthwhile to examine the potential effects of antidepressants, mood-stabilizers, and other psychoactive drugs on DNMT expression and function in immature and mature animal brain. These studies could provide a highly useful framework for the exploration of epigenetic mechanisms in major psychiatric disease, including suicide (Figure 1).

Poulter *et al.* (3) studied the frontal lobe of adult suicide victims. Perhaps the most interesting question that arises from this work relates to the timing of the observed *GABA-A a1* DNA hypermethylation. One possibility would be that this molecular alteration is the reflection of a suicidal "state" and not present in the time period preceding suicidality. However, it is equally possible that some of these DNA methylation events might have occurred during exposure to adversity or various stressors earlier in life, including childhood and adolescence, or even during

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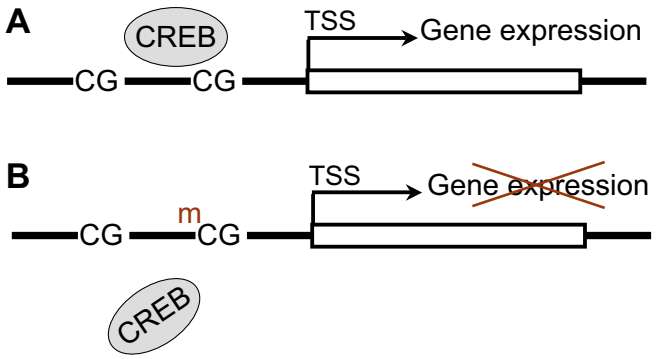

Figure 1. Epigenetic dysregulation of γ-aminobutyric acid (GABA)-A receptor subunit gene expression. Hypothetical scheme of *GABA-A a1* gene expression in prefrontal cortex of control subjects **(A)** and suicide victims **(B)**, based on Poulter *et al.* (3). In control subjects, CpG dinucleotides residing in the proximal *GABA-A a1* gene promoter remains mostly unmethylated, resulting in chromatin permissive for cAMP response element-binding protein (CREB) transcription factor binding and active gene expression from the transcription start site (TSS). In contrast, a subset of CpGs within the *GABA-A a1* gene promoter become methylated in suicide victims, resulting in repressive chromatin and lack of CREB binding and decrease or shutdown of gene expression.

intrauterine development. This hypothesis is especially interesting, given the already mentioned association between abuse and maltreatment in childhood and suicidal behavior in adulthood (1). I am not aware of studies that looked at developmental DNA methylation changes specifically of the GABA-A a1 gene in human brain. However, a closely related gene, *GABA-A a2*, shows robust and progressive DNA methylation increases across the full lifespan in human temporal cortex (7), and there is evidence that GABAergic signaling is epigenetically regulated throughout the extended period of normal prefrontal development (8). Therefore, one might speculate that alterations in DNA methylation and other types of chromatin modifications eventually could emerge as key events in a pathophysiological cascade originally triggered by adverse events and various stressors in childhood and adolescence, then impairing GABAergic and other neurotransmission and significantly increasing the risk for suicidal behaviors in the context of mood disorders or schizophrenia and other psychiatric disease.

Interestingly, a recent study, using a postmortem collection that is different from the one used by Poulter *et al.* (3), reported hypermethylation of ribosomal DNA promoter sequences in hippocampus of suicide victims with a history of childhood abuse or neglect (4). These changes occurred in conjunction with a deficit in ribosomal RNA expression in hippocampus. Because ribosomal RNAs encode the building blocks for the protein synthesis machinery of the cell, the hypermethylation of ribosomal DNA is likely to profoundly impair cellular functions in the affected hippocampi. Taken together, the two studies on suicide postmortem brain (3,4) provide evidence that DNA hypermethylation: 1) affects multiple brain regions, 2) involves both DNA repeats and single copy genes, and 3) might in some cases be related to childhood trauma.

Finally, it is worth mentioning that although DNA and histone modifications are traditionally viewed as the molecular basis of epigenetic phenomena, it is likely that the neurobiology of suicide includes additional mechanisms affecting gene expression and function in the absence of DNA sequence alterations.

A very striking example involves the increase in RNA editing of the serotonin 2C receptor (5-HT2C) gene transcript in prefrontal cortex of suicide victims, a molecular alteration not explained by comorbid psychiatric diagnoses (9,10). The *5-HT2C* pre-mRNA is among a limited number of gene transcripts targeted by adenosine deaminases that convert RNA adenosine residues to inosine, which leads to changes in the amino acid composition and downregulation of 5-HT2C receptor activity (9,10). This abnormal increase in RNA editing activity thus is thought to contribute to defective serotonergic signaling in the frontal lobe and possibly in other brain regions of suicide victims (9,10). Importantly, the brain of fluoxetine-treated animals shows *5-HT2C* RNA editing changes that are opposite to those observed in the suicide postmortem brain, which further underscores the potential significance of the RNA editing machinery for the pathophysiology and also pharmacological treatments of depression and suicidality (9).

One can anticipate that, following the aforementioned pioneering work, the study of DNA methylation, chromatin remodeling, and RNA editing in the suicide brain and in preclinical models will move to center stage.

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