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Major depressive disorder: understanding epigenetic basis for pharmacological care

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Abstract: Major depressive disorder (MDD) is a significant health burden that afflict men and women worldwide. Currently, the treatment options are limited with low efficacy. The underlying cause of MDD is largely unknown, but epidemiology studies have pointed to the influence of adverse life events and stressful experiences. In this review, we analyzed previous studies of MDD in both humans and animal models, and raise a hypothesis that stress-induced epigenetic changes may contribute to the pathogenesis of MDD. We propose that stressful experience triggers long-lasting changes in the epigenome, and thereby leading to enduring changes in gene expression that underlie the etiology of MDD. We next discussed current experimental paradigms to model stress-induced behavioral maladaptation in rodents, highlighted the top candidate epigenetic mark, and underscored the top brain region for future studies. In addition, we recommend the adaptation of recently developed CRISPR technology to modify the epigenome in order to assess the causal relationship between epigenetic changes and pathogenesis of MDD. We believe a better understanding of the underlying cause of stress-related neuropsychiatric disorders, such as MDD, will not only improve early diagnosis and intervention, but also reveal new targets and new directions for novel therapeutic development and pharmacological treatments.

Key Words*–Major Depressive Disorder, Stress, Epigenetics, DNA methylation, Prefrontal Cortex*

I. INTRODUCTION

Current epidemiological studies have found that stress is a key determinant of susceptibility to major depressive disorder (MDD) [1-3]. Worldwide, MDD affects about 6% of adults in their lifetime and represents a significant health burden, costing the economy billions of dollars each year [4,5]. Despite this, currently available antidepressant therapies are ineffective in nearly half of the patients, emphasizing the need for additional treatment options [6,7].

Given that stress is a common factor in the etiology of MDD, understanding the molecular mechanisms that convert stressful experiences into long-lasting changes in physiology and behavior is of crucial importance for understanding MDD pathogenesis and for developing much-needed therapeutic options. In this review, we discuss the concept of epigenetics that mediates stress-induced long-lasting changes in gene expression relevant to the development of depressive behaviors. Epigenetics refers to the study of stable alterations in gene expression potential without changes in the underlying DNA sequence [8]. Epigenetic changes encompass an array of molecular marks on both DNA and chromatin, including DNA methylation and histone modifications [9-11]. Notably, epigenetic modifications are relatively stable and can be inherited mitotically in somatic cells and meiotically in germ cells [12]. This heritability suggests a mechanism by which the environment, or experience, interacts with the genome to affect long-lasting physiological and behavioral outcomes. Epigenetic modifications are also reversible and prone to adjust upon enduring changes in environment or experience, thereby making them ideal targets for drug development.

Thus, understanding the epigenetic basis of stress-related MDD by 1) identifying the "epigenetic code" associated with stress, and 2) proving the causality of this epigenetic code to increased risks of MDD development, will provide a critical foundation for the identification of new drug targets and development of novel therapeutics.

II. HYPOTHESIS

A growing body of evidence from animal and human studies has implicated adverse environments in altering epigenetic programming of gene expression and promoting maladaptive behavior [3]. One notable example is chronic social defeat in adult mice was found to induce the manifestation of depression-like behaviors that are mediated by decreased DNA methylation at the promoter of *corticotrophin-releasing factor* (*CRF*), leading to increased *CRF* expression [13]. Interestingly, increased CRF concentrations in cerebrospinal fluid (CSF) have been repeatedly observed in MDD and suicide victims [14,15]. It has been speculated that

stressful events trigger decreased DNA methylation at the *CRF* promoter, resulting in persistently elevated CRF concentration in CSF. Several other genes, including *Gdnf* [16], *Bdnf* [17], *Mecp2*[18], *and b-catenin* [19], haves also been implicated in mediating the effects of stress on behavioral maladaptation.

We hypothesize that stress is encoded, at least partially, in an "epigenetic code" that regulates long-lasting transcriptional outcomes underlying clinically depressive behaviors. We define this code as a list of differentially methylated regulatory regions of the genome crucial for the control of downstream genes that have been or predicted to be functionally linked to depression or depression-like phenotypes. In the next session, we discuss the priorities to experimentally model MDD in rodents, highlight the focus of candidate epigenetic marks and brain regions, and finally outline a novel strategy to assess the causal role of "epigenetic code" to the development of depressive-like behaviors.

III. METHODS OF CHOICE

3.1 Choice of Stress Paradigms

Men and women manifest different symptoms and coping responses to episodes of depression and respond differently to stress [20.21]. Sex-specific differences in stress response are also observed in mice [22]. Thus, sex-specific stress paradigms have to be applied in order to model stress in males and females independently. In the field of MDD research, various forms of stress paradigms have been studied to induce behavioral maladaptation relevant to depression. Among these, one of the most robust and widely used models is chronic social defeat [23,24]. Social stress in rodents is projected to reflect human clinical situations, and thereby promotes anxiety and social withdrawal. Male mice are territorial animals that will attack conspecific intruders. Taking advantage of this behavior, the social defeat paradigm involves placing a male experimental mouse in the home cage of a territorial aggressor that will physically overpower the intruder. Between bouts of defeat, intruders and aggressors are housed in the same cage and separated by a perforated divider, exposing the experimental mouse to further psychological stress. Several bouts of defeat are sufficient to produce a robust, depression-like phenotype marked by anhedonia, anxiety, and social-avoidance in stress 'susceptible' mice, in contrast to a small minority 'resilient' to the social stress. Previously published reports have shown that while undefeated control mice spend much of their time interacting with a novel mouse, susceptible animals spend markedly less time in close proximity to an unfamiliar animal. This behavior has been shown to be long lasting with defeated mice displaying social withdrawal when tested several weeks later [24].

The social defeat paradigm, however, cannot be applied to female mice. Despite the fact that depression is far more prevalent in women [25,26], most of the stress-related depressive behavioral studies to date have been characterized in male rodents. Recently, Russo and colleagues adapted and applied a subchronic variable stress (SCVS) model in female mice to produce consistent coping behaviors, measured by novelty suppressed feeding, sucrose consumption, and formed swim test, similar to depression-like behaviors [27]. The SCVS paradigm consists of 6 days of alternating stressors to prevent stress habituation. They found that this set of stressors was sufficient to induce coping behaviors in female but not in male mice. Thus, these studies support that social defeat and SCVS can be used to model stress-related behavioral maladaptation in male and female mice, respectively.

3.2 Choice of Epigenetic Marks

There are multiple types of epigenetic modifications have been reported in studies covering stress-related development of depression-like phenotypes in rodents and MDD in humans. These include specific histone marks such as acetylation, methylation, and phosphorylation; specific incorporation of histone variants; and the alteration of short and long non-coding RNAs [28]. Other studies have also found that DNA methylation, an epigenetic modification on cytosine, plays a similar imperative role in mediating stress responses. For example, several studies have shown that early life stress in rodents [29,30] and humans [31,32] induces changes in DNA methylation at a few tested gene promoters. Methylation of DNA at regulatory regions of the genome (REGs), such as enhancers and promoters, is thought to inhibit downstream gene transcription by interfering with transcription factor binding and/or by recruiting methyl-CpG binding domain proteins (MBDs) that alter transcription efficiency [12]. Considering the relatively stable nature of DNA methylation, this has been proposed as a leading mechanism in mediating long-term changes in gene transcription and animal behavior.

In mammals, DNA methylation mostly occurs at CpG dinucleotides (mCG). Recent studies, however, have demonstrated that methylation can also occur in the CA context (mCA), and that this type of methylation is selectively enriched in the brain and developmentally up-regulated at the time of synaptogenesis in both mice and humans [33,34]. Furthermore, DNA methylation, once thought as a permanent epigenetic mark throughout life, has recently been found to be able to be modified through oxidization into hydroxymethylcytosine (hmC), leading to erasure of the methylation mark. This process has been described as a DNA demethylation pathway. Similarly, hmC is found to be particularly abundant in brain tissue compared to peripheral tissues, implicating

the dynamic nature of DNA methylation in the brain [35-37]. Thus, the stability of DNA methylation and its reversible characteristics have made it an ideal candidate to encode the molecular memory of experiences.

The importance of DNA methylation to mental health is further underscored by the evidence that changes in DNA methylation levels have been linked to susceptibility to psychiatric conditions including MDD [38]. Furthermore, human genetic studies have implicated mutations in the writers (DNA methyltransferases, DNMT1, 3a and 3b), editors (methylcytosine dioxygenases, TET1-3), and readers (methyl-CpG binding domain proteins, MBDs) of DNA methylation in a variety of disease conditions, including neuropsychiatric disorders [28]. Single nucleotide polymorphisms in the human *DNMT3a* gene are also found associated with stress-induced negative emotional responses [28]. Given all of these reasons, alteration of DNA methylation at selective REGs holds the potential to regulate enduring changes in gene expression responsible for depression-like phenotypes.

3.3 Choice of Brain Regions and Cell Types

The brain is an astoundingly complex organ comprised of discrete areas, different neuronal cell types, and distinct neural circuits that execute various biological functions [39]. In response to stressors, animals, like humans, adopt either adaptive or maladaptive behavioral strategies. Previous studies have found that the prefrontal cortex (PFC) is required for the executive control of behaviors and for effective coping with stress [40,41]. Clinical studies have also implicated PFC dysfunction in MDD and other anxiety-related disorders [42-44]. A number of structural and functional changes in pyramidal neurons of the medial PFC (mPFC), such as dendritic remodeling, spine loss, and altered synaptic transmission, have been reported in animals experiencing sustained stress exposure [45-48]. Thus, excitatory pyramidal neurons in the PFC appear to be particularly relevant to stress experience.

IV. ASSESSING CAUSALITY

Epigenetic modifications, in particular DNA methylation which modifies cytosine to regulate enduring programs of gene expression, have been increasingly associated with depression-related behavioral abnormalities in animal models and MDD patients [28]. Causality between these modifications and depression, however, has yet to be established. This is partially due to the limited technology available to modify DNA methylation in a locus-specific manner. Previous methodologies to alter the epigenome by increasing or decreasing the expression levels of DNA methyltransferases or histone deacetylases led to global changes in the epigenome and transcriptome, but lacked locus specificity [49]. The development of programmable DNA-binding proteins such as zinc finger proteins (ZFPs) [50] and transcription activator-like effectors (TALEs) [51,52] makes locus-specific epigenetic modification feasible [53], but this approach is costly and time consuming. In the past, many studies have also focused on direct manipulation of the stress-responsive genes, using overexpression, knockdown or knockout approaches, to assess their roles in stress-induced behavioral adaptation. The overexpression, knockdown or knockout approaches, however, often lead to dramatic changes in gene expression levels. This is typically not happening under *in vivo* situations. Therefore, an innovative approach is needed to overcome these technical and conceptual limitations.

Recently, the expansion of CRISPR/Cas9-based genome engineering technology[23] has made epigenome editing possible. CRISPR/Cas9 is a versatile technology for genome engineering first discovered in microbes [54]. The Cas9 nuclease can be directed to a specific genomic locus via complementary binding of an engineered guide RNA (gRNA) and the target site [55]. The enzymatic activity of the Cas9 nuclease can be abolished by mutating its RuvC and HNH domains, generating a nuclease-null deactivated Cas9 (dCas9) [56,57]. Recent studies have shown that upon fusing dCas9 to a gene repression domain or an activation domain, dCas9 can function as a synthetic transcriptional repressor or activator, respectively [58]. Similarly, fusing dCas9 to the catalytic domain of histone acetyltransferase p300 [59], histone demethylase LSD1 [60], DNA methyltransferase DNMT3a [61,62], and methylcytosine dioxygenase TET1 [61,63,64], results in an increase of histone acetylation, loss of histone methylation, increase or decrease DNA methylation, respectively, at gRNA-directed genomic loci.

Importantly, the CRISPR/Cas9 platform overcomes all of the above limitations [57]. Given the easily programmable nature of Cas9/gRNA and its application potential in epigenome engineering, we recommend adapt CRISPR/Cas9 and develop it as a versatile tool to alter DNA methylation in a locus-specific manner. Therefore, once a list of epigenetic code is identified, CRISPR-based epigenomic editing will allow, for the first time, the test of causality of this epigenetic code to the development of depressive-like behavioral phenotypes.

V. CONCLUSIONS

Overall, in order to advance our currently limited understanding of the epigenetic mechanisms underlying stress-related high risks of MDD development, the identification of an epigenetic code associated with stress, the adaptation of CRISPR/Cas9 technology to modify locus-specific DNA methylation, and the

assessment of a causal relationship between epigenetic changes and behavioral alterations, are all required to provide an important foundation for ultimately identifying robust biomarkers and developing novel therapeutics. The discovery of new targets is not only important for development of therapeutics targeting MDD, but also other psychiatric disorders sharing similar etiologies, such as bipolar disorder, post-traumatic stress disorder (PTSD), and schizophrenia.

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