Epigenetic Approach to PTSD: In the Aspects of Rat Models

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Abstract

Posttraumatic stress disorder (PTSD) is a stress-related mental disorder and develops after exposure to life-threatening traumatic experiences. The risk factors of PTSD included genetic factors; alterations in hypothalamic–pituitary–adrenal (HPA) axis; neurotrophic, serotonergic, dopaminergic, and catecholaminergic systems; and a variety of environmental factors, such as war, accident, natural disaster, pandemic, physical, or sexual abuse, that cause stress or trauma in individuals. To be able to understand the molecular background of PTSD, rodent animal models are widely used by researchers. When looking for a solution for PTSD, it is important to consider preexisting genetic risk factors and physiological, molecular, and biochemical processes caused by trauma that may cause susceptibility to this disorder. In studies, it is reported that epigenetic mechanisms play important roles in the biological response affected by environmental factors, as well as the task of programming cell identity. In this article, we provided an overview of the role of epigenetic modifications in understanding the biology of PTSD. We also summarized the data from animal studies and their importance during the investigation of PTSD. This study shed light on the epigenetic background of stress and PTSD.

Keywords
► PTSD
► epigenetics
► methylation
► rat studies

Introduction

Posttraumatic stress disorder (PTSD), which is among the stress-related mental disorders, develops after exposure to life-threatening traumatic experiences. The main risk factor of PTSD is genetic factors which are affected by many important biological systems, such as the hypothalamic–pituitary–adrenal (HPA) axis; neurotrophic, serotonergic, dopaminergic, and catecholaminergic systems; and a variety of environmental factors, such as war, accident, natural disaster, pandemic, physical or sexual abuse, that cause stress or trauma in individual.[1–3]

Exposure to trauma varies from person to person, depending on many interacting risk factors, including gender, genetics, and social interaction, and early life experiences.[4,5] As a result of this difference, the individual may not develop PTSD, or the symptoms seen in people who develop PTSD may spontaneously improve or, on the contrary, the individual may be at high risk of recurrence of symptoms, even though they have recovered from PTSD.[6] The fact that some individuals are still in the high-risk group for PTSD despite the decrease in symptoms emphasizes that the effects of long-term processes should be taken into account in the response to trauma. Therefore, when seeking a solution for

PTSD, it is important to consider preexisting genetic risk factors and physiological, molecular, and biochemical processes caused by trauma that may cause susceptibility to this disorder. Although it is an effective method to use patients while investigating human diseases, it is not ethically appropriate to induce PTSD in healthy individuals and the occurrence of mental disorders such as PTSD in humans is not always real-time but often coincidental.[7] Since of all these cases that limit the use of human subjects, rodent animal models are generally accepted and used by research groups in PTSD studies.

Existing Rodent Models of Inducing Symptoms Seen in People with PTSD

Present animal models fall into three different categories to induce symptoms seen in people with PTSD; it uses physical, social, and psychological stress factors, and these stress factors can reveal common or differentiated neuroadaptation patterns in all models.[8] In physical stress administrations, different stressors are used either alone or in combination to evaluate behavioral responses to stress. The time-dependent sensitization model which is based on sensitivity, mainly uses psychological or pharmacological stressors. The common feature of all these experiments is sensitization. A weak stimulus given after a dangerous stimulus causes an increase in sensitivity, although it is less than the intensity of the life-threatening stimulus. Under natural conditions, sensitization is a principal action to threat as it accelerates escape responses and thus may protect the subject from future danger.[9] Underwater trauma model is based on forced to swim for 1 minute, then held underwater during 30 seconds. Foot shock is a model in which the electric shock (in different intensity and duration) applied to the foot of the animal placed in the apparatus is used as a physical factor.[10] With the foot shock model, avoidance and anxiety behavior can reproduce some of the main symptoms of PTSD, including hyperarousal, aggression and flashback, and sleep disturbance.[10,11] Several studies are used auditory or visual stimuli (situational reminder [SR]) in addition to the classical fear conditioning procedure such as foot shock, using these stimuli given with shock to animals to remember fear aftershock. Unlike the classical model, in the Stress-Enhanced Fear Learning (SELF) model, animals are either not exposed to any shock or are exposed to unpredictable foot shocks many times in a single session.[12] One of the most widely used PTSD models is the single prolonged stress (SPS) model. In this model, rats are exposed to varying stress factors. In the general SPS protocol, the animal is kept in a restriction cage for 2 hours, then subjected to forced swimming and exposed to diethyl ether until unconscious.[13] The restraint/immobilization stress model, also used as part of the SPS procedure, is applied by restraining the animal using plastic retention tubes or immobilizing the four limbs and head on a board in a prone position.[14] It is also possible to benefit from the natural social behaviors of animals with social stress factors, instead of directly stressful stimulants. Since humans are known to develop PTSD in response to social experiences, such as rape and childhood abuse, it is reasonable to think that this applies to another species as well.

PTSD models which are using social stressors are often used in conjunction with other PTSD psychological models. The housing instability (HI) model is applied by placing the animal randomly in cages with different animals each time.[15] This model makes sense considering that PTSD patients are affected by HI.[16] Animals subjected to this model are generally exposed to the psychosocial stress model at first. As a psychosocial stress model, predator-based models are generally used. The predator-based model is based on the hypothesis that a synergistic effect develops due to the interaction of risk factors and PTSD. Applied at different times and it includes exposure to predator stressor, random cage change, and immobilization stress application procedures.[15,17] In predator-based models, animals are exposed to either the predator itself or a clue of the predator, such as urine, faces, or feathers. Studies have shown that rats have a strong innate fear of a predator.[18] There are many studies in the literature in which trimethylthiazolin (TMT), a synthetic compound isolated from other predator odors and urine, is used instead of living animals.[19] The Predator Scent Stress is the model in which animals are exposed to the scent of their natural predator to model how humans reproduce variations in response to trauma. For example, stress group rats are exposed to dirty contaminated cat litter for 10 minutes, while rats in the control group are exposed only to clean litter for the same period. On the last day when the behavioral parameters of the animals will be evaluated with a maze (elevated plus, T or radial) or/and open field test mechanisms, animals in all groups (both stress and control groups) are exposed to clean cat litter for 10 minutes, creating a situation that reminds trauma and reexperiencing trauma.[20]

Recall of traumatic memories, which are among the symptoms of PTSD, is directly related to dysregulation and deterioration in the reconstruction of fear memory.[21] Moreover, traumatic events can also affect memory formation as they often marked strong memories. Therefore, PTSD models which using fear conditioning is focused on the processing of fear memory, behavioral responses, physiological, molecular, and neuro epigenetic processes in the basic brain regions responsible for fear learning and memory processes such as the hippocampus, amygdala, and frontal cortex.[21–23]

After different combinations of procedures, animals display impaired habituation behaviors in their new environment.[24] It is also possible to induce PTSD-like symptoms using social isolation and maternal separation (early life stress model) such as the random cage change made in the HI model.[25,26] In the social defeat-SD model, which is used to trigger-off avoidance behavior among PTSD symptoms, subjects are exposed to a single aggressive animal.[27,28] Although both physical and social stress factors produce behavioral and neuroendocrine changes observed in PTSD, most of the relevant models do not take into account that people show different responses to trauma, some individuals are sensitive to PTSD, and some are resistant. Psychological stress, which

elicits instinctive responses in animals to natural predators, more successfully reveals the difference between those susceptible to the development of PTSD and resistant ones.[7]

The models that follow the provocation of fear and stress in animals are based on the elimination of the stress factor and the comparison of recovery and related biological responses with homeostasis. Animal models of PTSD can mimic human symptoms quite well, depending on the conditioning or triggering stressor factor. Unfortunately, animal models cannot explain well the differences between individuals in fear acquisition and disappearance of fear.[29,30]

PTSD models that expose animals to the factors of trauma before fear conditioning may be said to have greater validity than fear conditioning alone. To be able to determine the epigenetic effect of trauma and stress subsequently, fear-learning stress and/or trauma-enhanced fear learning models has been used. These studies have been provided to define the important role of drain-derived neurotrophic factor (BDNF) on stress and fear memory. According to the results of the SEFL paradigm study, the first response is included the hippocampal BDNF/TrkB signal to fear to condition which increases exon I and IV BDNF mRNA levels, acetylation in H3 and H4 histones, and TrkB protein levels in the exon I and IV BDNF promoter regions. BDNF, which is considered to be a key regulator of the development of neurons in the central nervous system, is thought to be an important factor mediating synaptic plasticity.[31,32]

There is accumulating evidence in the literature that traumatic experiences are associated with epigenetic modifications in the human genome.[33] Epigenetic modifications are associated with alteration in gene expression. Also, the epigenome is one of the susceptibility factors which contributes to PTSD.[33,34]

Epigenetic Alterations in Posttraumatic Stress Disorder

Genetic and environmental factors play a key role during the development of PTSD. Advancement of molecular and genetic diagnostic methods help identification of genetic alterations of PTSD.[34] Studies highlighted that parental exposure to posttraumatic stress can cause potential biological alterations in their offspring.[35,36] These studies show the importance of the heritability of PTSD and paved the way for genetic studies. Clinical diagnosis of PTSD is based on behavioral symptom clusters that are most directly associated with brain function, epigenetic studies of PTSD in humans have been limited to peripheral tissues such as blood, buccal tissue, and saliva. Animal models of PTSD, predominantly in rodents, have been used for the identification of the epigenetic alterations in the brain.

Epigenetic modifications are effects on gene expression status without altering nucleotide sequence. Epigenetic modifications involve DNA methylation, histone modifications, RNA modifications, and noncoding RNAs. Epigenetic alterations are influenced by both environmental and genetic factors. Epigenetic alterations are under control by several enzymes, like DNA methyltransferases, DNA demethylases,

lysine methyltransferases and demethylases, and so on. The reversible nature of the epigenetic alterations is the crucial point of these modifications, and this makes them a potential target during the evaluation of disease and as a target for the treatment planning.

Histone Modification

Histone proteins are important during the packaging of DNA and directly related to gene regulation. Histones chemically modified through the action of enzymes to regulate gene transcription. Histone acetyltransferase (HAT) is responsible for histone acetylation and acetylation of histones causes the noncondensing or loosening of DNA which allows transcription. Histone deacetylase (HDAC) is responsible to remove the acetyl group from histones and causes the coiling of DNA, creating condensed densely packed DNA which inhibits or repress the gene expression. Histone modifications are attached N-terminal tail of histones covalently. These modifications are involved acetylation, methylation, phosphorylation, ubiquitination, and sumoylation.[37] Lubin and Sweatt demonstrated that a global increase in histone H3 acetylation and phosphorylation was related to recalling of fear memory in the CA1-4 region of the hippocampus[38] and Maddox and Schafe showed recalling fear memory promoted increased level acetylation of histone H3 in the lateral amygdala.[39] In a study using a rodent model of PTSD, it was shown that histone hyper-acetylation was triggered in LA after ingestion of fear memory inhibitors.[40]

Studies demonstrated strong interaction between fear and histone acetylation.[41,42] Webb et al demonstrated the interaction between fear memory and H3K4me3 and 5-hydroxymethylcytosine (5-hmc) in the hippocampal CA1 region and anterior cingulate cortex (ACC).[42] Therefore, these studies show us the interaction between epigenetic alterations and their effects on different brain region during fear memory formation. Takei et al used an SEFL paradigm with an SPS in rats and observed an increased level of acetylation of histone H3 and H4 at the exon I and IV BDNF promoter regions in SPS rats in their hippocampus.[43] Takei et al demonstrated enhanced hippocampal BDNF/TrkB signaling in response to fear conditioning in this animal model of PTSD.[43] The reversible nature of epigenetic modifications makes them a therapeutic target. HDAC, DNA Methyltransferases (DNMT), and Histone methyltransferases (HMT) inhibitors are widely used inhibitors in clinical practice and researches which changes the effects of modifications especially in cancer. Studies emphasized that drugs targeting HDACs and DNA methyltransferase inhibitors as an emerging anticancer strategy and The Food and Drug Administration (FDA) has approved these agents as a treatment target. Lee et al demonstrated that knockdown of HDAC6 prevents the enhancement of glutamatergic signaling by acute stress in rats.[44] Studies demonstrated that HDAC inhibitors sodium butyrate (NaB), trichostatin A, and valproic acid which elevate histone acetylation levels, has therapeutic effects in rodent stress paradigms, improving cognition, and reducing anxiety behaviors.[45]

DNA Methylation

DNA methylation is one of the most studied epigenetic alterations. Methyl groups bind covalently fifth carbon of cytosine nucleotides on DNA. DNA methylation is a reversible process and DNA methyltransferases are responsible for adding methyl groups and DNA demethylases are responsible for removing those methyl groups. Methylation of cytosine nucleotide is directly related to gene expression. DNA methylation is important during tissue differentiation, tissue-specific gene expression, and X inactivation.[46] Advancement of diagnostic tools will help identification of different DNA modifications, like 5-methylcytosine (5-mc) and 5-hmc.[37,47] These modifications are important in PTSD and learning.

Methylation studies showed that FK506 binding protein 51 gene, *BRSK1*, *LCN8*, *NFG*, *DOCK2*, *ZFP57*, *RNF39*,[46–48] *BDNF*, *NR3C1*, *MAN2C1*, *TLR8*, *SLC6A4*, *IL-18*, *SKA2*,[3] *LINC01090*, *BC036345*, *ZNRD1-ASI*, and *RORA*,[49–55] *TPR*, *ANXA2*, *CLEC9A*, *ACP5*, *TLR8*, *LRRC3B*, *BRSK1*, *LCN8*, *NGF*, *DOCK2*,[48] *DUSP22*, *HIST1H2APS2*, *HOOK2*, *NINJ2*, *PAX8*, *RNF39*, *ZFP57*,[56] *NRG1*, *HGS* genes are related with PTSD and severity of symptoms[48,56,57] (►Table 1). The FK506-binding protein 51 (*FKBP5*) gene is one of the genes on HPA axis and an important regulator of stress response through altering glucocorticoid receptor sensitivity.[58] Klengel et al identified an interaction between demethylation of FKBP5 and early trauma exposure.[55] Rodent studies demonstrated that parental care influences revealed that differences in maternal

phenotypes effects their pup's development of behavioral and HPA responses to stress as adults.[59] Elevated level DNA methylation was identified in the offspring after low maternal care and lower methylation was identified in high maternal care.[59,60] Li et al demonstrated the interaction between fear extinction and Tet3-mediated global 5-hmc in the infralimbic region of the ventromedial prefrontal cortex.[61]

Studies highlighted that different brain regions, like dentate gyrus (DG), dorsal DG, dorsal CA1, ventral CA3, basolateral amygdala, and medial prefrontal cortex, show different level of gene expression during the stress induction.[62] Roth et al reported that in PTSD, BDNF exon IV was hypomethylated in the ventral CA3 and no methylation changes observed in other hippocampal subregions in rats.[63] It is also reported in the literature that there is a relationship between the epigenetic status of catechol-O-methyltransferase and PTSD.[64]

NR3C1 is another most studied gene which encodes the GR. *NR3C1* plays a key role in the understanding of the epigenetic regulation of PTSD and fear extinction processes.[65] As a result of the study examining the relationship between the methylation level of the *NR3C1* promoter and PTSD, it is emphasized that the higher level of DNA methylation in the NR3C1 promoter region could be a protective effect against vulnerability to PTSD.[66]

HDAC4 gene was related to learning and memory-related processes and studies demonstrated that higher level

Table 1 Differentially methylated human genes in PTSD

Gene	Tissue type	Species	Methylation status	Reference
BRSK1	Blood	Human	Hyper	46–48
LCN8	Blood	Human	Hyper	46–48
NFG	Blood	Human	Hyper	46–48
DOCK2	Blood	Human	Hyper	46–48
ZFP57	Blood	Human	Hyper	46–48
RNF39	Blood	Human	Hyper	46–48
NR3C1	Whole blood	Human	Hypo	3
MAN2C1	Whole blood	Human	Hypo	3
TLR8	Whole blood	Human	Hypo	3
SLC6A4	Whole blood	Human	Hypo	3
IL-18	Whole blood	Human	Hypo	3
SKA2	Whole blood	Human	Hypo	3
DUSP22	Blood	Human	NS	49
HIST1H2APS2	Blood	Human	Hypo	49
HOOK2	Blood	Human	NS	49
NINJ2	Blood	Human	NS	49
PAX8	Blood	Human	NS	49
RNF39	Blood	Human	Hypo	49
ZFP57	Blood	Human	Hypo	49

Abbreviations: Hyper, hypermethylation in case relative to control; Hypo, hypomethylation in case relative to control; NS, nonspecified; PTSD, posttraumatic stress disorder.

methylation was observed in PTSD cases.[63] In the study where *BDNF*, *NR3C1*, *MAN2C1*, *TLR8*, *SLC6A4*, *IL-18*, and *SKA2* gene methylation analyses were performed in patients suffering from PTSD, it was concluded that BDNF, NR3C1 and MAN2C1 methylations are associated with PTSD diagnosis.[3] This study revealed the importance the regulation of genes involved in synaptic plasticity and the HPA axis and their association with PTSD.

Noncoding RNA

Noncoding RNAs (ncRNAs) are not translated into a polypeptide. They play a key role during the processing and regulation of other RNAs such as messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).[67,68] Recent studies showed the importance of ncRNAs as a biomarker for trauma-related brain disorders.[69] Ambeskovic et al demonstrated how transgenerational and multigenerational stress factors regulate cortical miR-221 and miR-26 expression and their target genes, ntrk2, and map1a, and crh in the rat. They concluded that early life experiences and prenatal stress are crucial factors for brain development and mental health.[70]

Maurel et al studied miR-15a-5p, miR-497a-5p, miR-511–5p, and let-7d-5p in brain areas of mouse model of PTSD.[71] They identified lower transcript levels of miR-15a-5p, miR-497a-5p, and miR-511a-5p in the hippocampus and hypothalamus and in the medial prefrontal cortex, downregulation of miR-15a-5p, miR-511–5p, and let-7d-5p. They concluded that miRNA expression in the different brain areas correlated to miRNA-based epigenetic modulation in stress-induced phenotypes.[71]

Li et al demonstrated that exposure of acute traumatic stress in early adolescent caused permanent changes in neural network and altered expression of CRFR1 and CRFR1 mRNA and miR-34c expression in Wistar rats.[72] These studied showed the importance of identification novel non coding RNAs as a candidate for biomarkers of PTSD.

Conclusion

PTSD characterized by systemic dysregulation in anatomical sites outside of the brain, maladaptive alterations of the HPA axis, and sympathetic nervous system sensitivity/responsivity, and alterations in neuroimmune dynamics.[59] Human-based epigenetic studies of PTSD demonstrated alterations in age-related CpGs, DNA methylation, alteration in estrogen-responsive genes, alterations in the dopaminergic system, and alterations in the HPA axis. Environmental factors may have long-term consequences on brain behavior as a result of interaction with the genome by regulating epigenetic mechanisms. The susceptibility of genes due to stress and/or trauma, the effect of epigenetic changes on the development of PTSD has recently become more remarkable. In the literature, methylation analyses were performed using genes encoding proteins, such as BDNF, FKBP5, IL-18, MAN2C1, NR3C1, SKA2, SLC6A4 and TLR8, which play a role in the synaptic plasticity, immune system, serotonin modulation, neurogenesis, and inflammation response in PTSD.[3,73] While clinical researches are vital in the

administration of new therapeutic agents to people with PTSD, animal models are indispensable for comparing the behavioral and physiological effects caused by trauma, as well as molecular and biochemical abnormalities and the state of homeostasis.

Epigenetics studies provide a crucial role in understanding the interaction of epigenetics with environmental exposure to trauma in PTSD. Further studies will shed light on the interaction between stress-induced epigenetic regulations of neuronal function. The reversible nature of epigenetic modifications promises for determination of the epigenetic architecture of PTSD and may play a crucial role during the discovery of novel targeted therapeutic approaches to develop treatment strategies and prevention of PTSD. These studies will shed light on resolving several issues, like epigenetic treatment, trauma, or stress memory. Studies in the field of epigenetics will help to discover a marker for susceptibility to PTSD.

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