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## MicroRNAs as Biomarker and Novel Therapeutic Target for Posttraumatic Stress Disorder in Veterans

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Biomarker; miRNA; PTSD; Combat Veterans; post-traumatic stress disorder

### Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric disorder or mental illness that can develop upon exposure to or witnessing of a traumatic event. PTSD was first officially recognized in the *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed.(DSM III) in 1980 as an anxiety disorder (American Psychiatric Association, 1980). Since then, a wealth of knowledge has assembled regarding symptoms, epidemiology, assessment, subtypes, and treatment of this disorder. This has led to the transition in the fifth edition of the DSM (DSM-5), of PTSD to a trauma- and stressor-related disorder (American Psychiatric Association, 2013). The revised criteria reflect four symptom clusters of intrusive reexperiencing (i.e., flashbacks, unwanted memories), avoidance (i.e., avoiding reminders of the trauma), negative alterations in cognition and mood (i.e., thoughts of self-blame, difficulty experiencing positive emotion) and arousal (i.e., hyperarousal, difficulty sleeping)(American Psychiatric Association, 2013). The individual must experience all of these symptom clusters for duration of at least one month following direct or indirect exposure to actual or threatened death, serious injury, or sexual violence and be associated with distress or impairment in one or more areas of functioning to meet diagnostic criteria for PTSD. For Veterans, deployment for an extended period of time itself is a stressor and, coupled with high rates of combat exposure that are likely to occur, is a high-risk factor for PTSD. This disorder is frequently observed with other psychiatric diagnoses and traumatic brain injury (TBI) along with other comorbid symptoms like depression, particularly in military populations (Jaffee and Meyer, 2009; Tanev et al., 2014; Moore et al., 2014). Underscoring a need for future study in military and Veteran populations, servicemembers from the most recent conflicts show a prevalence of PTSD 2.5x greater than the general population (Fulton et al., 2015) and are less likely to benefit from first line treatments (Straud et al., 2019).

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PTSD prevalence rates have been reported to be 13.8% among Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) Veterans (Tanielian and Jaycox, 2008), 12.1% in Gulf War Veterans (Kang, 2003) and 15.2 % in male Vietnam War Veterans (Kulka et al 1990), which is greater than the 12-month prevalence in the US of 4.7% (Kilpatrick et al., 2013). Importantly, it is noted that the symptoms may not appear for several months to years after trauma exposure (Seal et al., 2008; O'Toole and Catts, 2017). Moreover, PTSD is associated with a host of other comorbidities including hypertension (Howard et al., 2018), cardiovascular disease (Dyball et al., 2019), cardiometabolic disease (Levine et al., 2014), suicidal thoughts, *etc.* (Millner et al., 2019) in addition to chronic pain (Toblin et al., 2014). Further elucidation of these comorbidities suggests poor long-term outcomes through several interacting pathways, including alteration in mental health, sociodemographic adjustments, health behavior, *etc.* (Ramsey et al., 2017). Consequences of PTSD for Veterans include increased healthcare utilization, decreases in functioning and increased risk for suicide (Marshall et al., 2000; Asnaani et al., 2014; Lutwak, and Dill, 2017), underscoring a need to understand the mechanisms of PTSD and inform effective interventions. Numerous predictors of PTSD have been identified, with peritraumatic factors (i.e., dissociation, life stress) being stronger than pre-trauma factors (e.g., education, prior trauma; Ozer et al., 2003, Brewin et al., 2000). More recently, biological factors have been associated with PTSD onset and treatment response. As examples, the inflammatory protein C-Reactive protein has been shown to prospectively predict PTSD among recently deployed marines (Early et al., 2014), and cortisol levels in response to waking predicted PTSD treatment response (Rauch et al., 2020). Thus, investigation of novel biomarkers is warranted to stretch our knowledge in PTSD and provide novel insights into possible biological mechanisms.

## Pathophysiology of PTSD

The pathophysiology of PTSD is complex as it covers vast functional aspects including noradrenergic, serotonergic, opioid, cannabinoid and hypothalamic-pituitary-adrenal (HPA) axis. PTSD is a neuropsychiatric condition derived from maladaptive alterations in neural plasticity including synaptic connection, dendritic remodeling, and neuronal growth which impacts neurocircuitry function and behaviors (Apfel et al., 2011). Functionally, the amygdala is the nodal point of fear regulation, and PTSD may evolve from hyperactivity of neurons in impaired amygdala (Helmuth L, 2003). In this review, we will briefly touch base of each aspect of pathophysiology in PTSD.

The adrenoceptor (AR) system is important in PTSD as it influences amygdala functioning (Strawn and Geraciotti., 2008). The AR system primarily activates CNS activity and stimulates sympathetic autonomic response in prefrontal cortex (PFC) and limbic systems resulting in the fear response (O'Donnell et al., 2004). Similarly, serotonergic receptors like 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>3</sub>, a group of G-protein-coupled receptors, are engaged in emotional and behavioral modulation (Cools et al., 2008). The opioid receptors ( $\delta$ ,  $\kappa$  and  $\mu$ ), a superfamily of G protein-coupled receptors, are implicated in etiology of PTSD (Dhawan et al., 1996). The  $\kappa$ -opioid receptor is particularly relevant to PTSD due to its expression in the PFC and cortex- hippocampal-limbic regions as well as associations with fear or anxiety related behavior (Bruchas et al., 2009). The endogenous cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub>) play a pivotal role in development of PTSD (Sbarski B., Akirav L., 2020). Among

the two receptors, CB<sub>1</sub> is well-studied at preclinical level and is of interest in PTSD. as it is distributed throughout the forebrain limbic structure (Leo and Abood, 2021) and modulates various behavioral issues including fear. Physiologically, PTSD affects multiple systems, including the HPA-axis, cortical function, and the immune system (Parsons and Ressler, 2013; MacNamara et al., 2016). A recent genome-wide association study of PTSD underscored the likelihood of genetic risk with schizophrenia, depressive-disorder, or bipolar depression (Duncan et al., 2018). It is suggested that three specific regions of the brain - amygdala, hippocampus, and PFC - are linked to fear memory or responsiveness in preclinical models and play a major role in the development of PTSD symptomatology ( Haubensak et al.,2010; Dieter, Engel, 2019; Andrewes et al., 2019; Henigsberg et al., 2019). The brain consists of several interrelated neural systems that activate and deactivate in response to different stimuli in a closely regulated way. Imaging studies showed patterns of dysregulation of both hippocampus and the medial PFC in patients with PTSD (Andrewes et al., 2019; Henigsberg et al., 2019; Bremner, 2007). Therefore, the neurobiology of PTSD is a complex process as exposure to traumatic events change neuronal morphology, function and neurochemistry (Cacciaglia et al.; Weiss, 2007).

In pursuit of biomarkers and a deeper understanding of PTSD pathophysiology, the role of micro ribonucleic acid (miRNAs), a class of non-coding RNAs, are emerging in psychiatric and neurological disorders, including schizophrenia, PTSD, anxiety, and major depressive disorder (Bartel, 2004). Deregulation of miRNA can impact the expression of multiple genes and their associated biological networks. Therefore, if it is supported that this is a novel molecular mechanism underling the pathogenesis of PTSD, the study of miRNA opens up a new area of investigation for novel therapeutic targets in PTSD. Therefore, investigating the role of miRNAs in the pathophysiology of PTSD using blood may provide a quick and an easy novel insight correlating the presence of disease. In this review article, we will describe miRNA biogenesis, miRNAs signature in Veterans with PTSD, HPA axis-FKBP5-miRNA-PTSD, epigenetic modification in PTSD, and conclude with future directions for study of miRNAs in PTSD.

## miRNA Biogenesis

Biogenesis of miRNA is an endogenous cellular process to generate mature and functional miRNA destined to target a specific messenger RNA (mRNA) for modulation. The miRNAs are a class of short noncoding regulatory RNAs, 21 to 23 nucleotides in length, that negatively regulate gene transcription through binding to the 3' untranslated region (UTR) of target mRNAs (Michlewski and Caceres, 2019; Alural B, Genc, 2017). The miRNA biogenesis pathway generates hundreds of unique miRNAs in mammalian cells. Each miRNA is capable of targeting hundreds of genes, thus simultaneously controlling multiple biological processes. It is thought that miRNAs regulate up to 60% of the protein-coding genome (Ruegger and Grosshans, 2012; Gregory et al., 2004). The biogenesis of miRNA starts in the nucleus. The miRNA is transcribed as a precursor molecule called primary transcript (pri-miRNA) by RNA polymerase III which turns it into a hairpin-like structure (Han et al, 2006). The nuclear event is catalyzed by a microprocessor complex that include a RNase III enzyme, Drosha, cofactors such as DGCR8 (DiGeorge syndrome critical region 8 gene) and associated proteins (Han et al, 2006; Ha M, Kim; Bohnsack et al., 2004). The

microprocessor complex cleaves the pri-miRNA into a 70-nucleotide-stem-loop precursor miRNA (pre-miRNA), which is subsequently exported to the cytoplasm by exportin-5 (Bohnsack et al., 2004; Hutvagner et al., 2001). Once in the cytoplasm, pre-miRNAs undergo a final processing by another RNase type III enzyme, DICER, to give rise to miRNA duplexes (Kobayashi and Tomari, 2006; Kwak and Tomari, 2012). Next, with another RNA binding protein like Argonaute 2(Ago2), the pre-miRNA is incorporated into the miRNA-induced silencing complex (miRISC), while the “passenger” strand is degraded with the formation of a mature, single-stranded ~21-nt-long miRNA (Han et al., 2006; Kobayashi and Tomari, 2006; Michopoulos et al., 2017). The strand recognition event is followed where the guide strand is recognized by “seed” sequence in the mature miRNA. The guide strand binds target gene and initiate mRNA degradation and translational repression. The mRNA degradation can be achieved by many mechanisms like binding to the 3'UTRs or the open reading frames (ORFs) of target genes leading to the degradation of target mRNAs or repression of mRNA translation (Fig. 1).

## miRNAs Signature in PTSD among Veterans

Since the discovery of miRNA, it has been established that the tiny molecule is highly conserved and suggested to be a key regulator in diverse physiological processes, including functioning of the nervous system. A large body of evidence elucidate the critical role of miRNA in psychiatric diseases (Issler and Chen, 2015), however, evaluation of the specific role of miRNA in Veterans with PTSD has more recently started emerging. On July 19 of 2021, a literature search using key words; miRNA, posttraumatic stress disorder, veterans (<https://pubmed.ncbi.nlm.nih.gov/?term=mirna+posttraumatic+stress+disorder+veterans&sort=date&size=50>) resulted in only 13 articles relevant to miRNA. Of these, 7 studies were original data articles examining miRNA in a sample of Veterans with PTSD. Currently, with the limited amount of scientific information available, we will illuminate the recent progress in miRNA dysregulation in Veterans with PTSD.

The first non-coding RNA snapshot was revealed in the peripheral blood mononuclear cells (PBMC) of OIF and OEF Veterans with and without mild traumatic brain injury (mTBI), approximately two thirds of whom also screened positive for PTSD (Pasinetti et al., 2012). Using Affymetrix Human gene 1.0 ST Array chip, authors have identified thirteen downregulated candidate small RNA biomarkers along with one miRNA, the miR-671-5p, in PBMCs of mTBI subjects (Pasinetti et al., 2012). Unsupervised clustering analysis further narrowed down to three small nucleolar biomarker panel; HBII-289, ENSG199411 and U35A which accurately selected mTBI from non-mTBI Veterans (Pasinetti et al., 2012). However, as both groups included Veterans with PTSD, no firm conclusions can be drawn about miRNA and PTSD. Presumably, the first miRNA landscape in combat Veterans with clinically diagnosed PTSD was reported in 2014 (Zhou et al., 2014). The study used a sample of combat Veterans returning from Persian Gulf, Iraq, or Afghanistan war who also had PTSD. Previous research has suggested that immune components contributed a pivotal role in PTSD (Kawamura et al., 2001; Jiang, 2008; Breen et al., 2015; Jones KA, Thomsen C., 2013); therefore, this study was aimed to determine the role of miRNA in immune dysfunction linked with PTSD. Using high-throughput miRNA microarray

hybridization analysis, the study investigated 1163 miRNAs in PBMC. Compared to control subjects, the PTSD group showed 7 upregulated miRNAs and 64 down-regulated miRNA (Zhou et al., 2014). The finding suggested significant alterations in miRNA expression corroborating with immunological changes, specifically enhanced pro-inflammatory Th1 and Th17 cytokine profile and decreased the regulatory T cells (Tregs) (Zhou et al., 2014). Together, the analysis showed that there was significant association between alterations in miRNA expression and immunological changes in combat Veterans with PTSD. Further, using the same Veteran cohorts, RNA-Seq on RNA samples from PBMCs of PTSD was performed by the same group. The study revealed 326 mRNA and 40 non-coding RNAs which were significantly altered in samples of those with PTSD compared to controls (Bam et al., 2016). Furthermore, a panel of downregulated miRNAs were identified associated with DNA methylation and immune deregulation (Bam et al., 2016). The study is interesting as the authors showed evidence of association between miRNA and DNA methylation and suggested that they play a critical role in immune system modulation in PTSD. Epigenetic changes in DNA methylation is an emerging concept and is associated with PTSD (Hammamieh et al., 2017). The authors have used extensive bioinformatic tools to dissect molecular signaling or pathways involved in PTSD pathology. Although promising, these findings need to be validated experimentally and in other cohorts.

Elevated level of pro-inflammatory cytokines has been observed in war Veterans with PTSD, suggesting a link between PTSD and inflammation (Gill et al., 2009). To determine the association between miRNA-mediated inflammatory response in PTSD, Bam M *et al* used PBMC samples from War Veterans of either 1991 Persian Gulf war, or Iraq or Afghanistan wars with PTSD, and age matched healthy controls. The authors showed that 183 miRNAs were downregulated that target several inflammatory genes in a first set of 4 control and 5 Veterans with PTSD (Bam et al., 2017). The observation was validated in an independent sample of 7 controls and 3 Veterans with PTSD by RT-PCR analyses and showed that JAK2, STAT1, IL23A, TGFB1, TGFB2, TGFB3, T-BET and CXCL3 were the predicted target for downregulated miRNAs. Furthermore, using healthy and PTSD patients' PBMCs, authors confirmed PTSD patients elicits more CD4+T cells that contribute to lowering miRNA expression (Bam et al., 2017). Mechanistically, authors demonstrated that inflammation in PTSD is partly due to the reduction of Argonaute 2 (*AGO2*) and Dicer1 (*DCR1*) elicits lowering the miRNA abundance and attenuation of *STAT3* transcript (Bam et al., 2017). The findings suggest that inflammation in PTSD could be the result of alteration in miRNA biogenesis components (*AGO2* and *DCR1*) and depletion of *Stat3* mRNA. An extensive study is warranted for targeting miRNA biosynthesis component(s), which may in turn inform therapeutic management in PTSD and inflammation.

Another study used peripheral blood samples of 24 returned military personnel from OEF/OIF conflicts with and without PTSD, to test for miRNA alteration (Martin et al., 2017). The miRNA sequencing analysis showed four upregulated miRNAs (miR-19a-3p, miR-101-3p, miR-20a-5p, and miR-20b-5p) and four downregulated miRNAs (miR-15b-3p, miR-125b-5p, miR-128-3p and miR-486-3p) expression in PTSD samples compared to those without PTSD (Martin et al., 2017). Furthermore, Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis predicted that these miRNAs are associated with axonal guidance and Wnt signaling in addition other physiological pathways at functional



standpoint (Martin et al., 2017). The Wnt or Wnt/ $\beta$ -catenin signaling, a highly conserved biological pathway involved in cell development, proliferation and fate and recently implicated to synaptic plasticity (Moon et al., 2004; Murase, Mosser and Schuman, 2002; Maguschak and Ressler, 2011; Maguschak and Ressler, 2012). Alteration of miRNA and Wnt signaling may provide a link to neurological process in the development of PTSD, but more studies are required to validate the findings.

An association between miRNA and Wnt signaling was further demonstrated in the PBMCs of Gulf war Veterans with PTSD. The authors demonstrated using RNA-seq and miRNA array analysis that a Wnt signaling component, the Wnt10b was upregulated (Bam et al., 2020). Wnt10b, a glycoprotein of Wnt family is known to contribute in cancer development (Milovanovic et al., 2004; Kharaihvili et al., 2011; Benhaj, Akcali and Oztuk, 2006). In the study, the authors showed that the miR-7113-5p was upregulated in the PBMCs, a bonafide candidate for epigenetic modification and inflammation (Bam et al., 2020). Interestingly, Wnt10b was shown to be associated with enhancing the proinflammatory response in PBMCs. Mechanistically, miR-7113-3p was found to be a target for Wnt10b and was significantly downregulated indicated an epigenetic modification and likely contributing the inflammatory response in PTSD (Bam et al., 2020).

Cell-free miRNAs circulating in the bloodstream have been found to be enclosed into extracellular vesicle (EV), called exosome (Raposo and Stoorvogel, 2013). Exosomes are emerging as a new communicating cellular vehicle in diverse biological processes including neuroinflammation and TBI (Raposo and Stoorvogel, 2013; Brites and Fernande, 2015; Andjus et al., 2020; Harrell et al., 2021; Guedes et al., 2020). Critically, exosomes transport miRNA. There is only one study that showed alteration of miRNA in the exosome of combat Veterans in EV and EV-depleted (EVD) plasma separately. Study showed that pattern of miRNAs was different between EV and EVD plasma among male OEF/OIF combat Veterans with and without PTSD (n = 12 each group) (Lee et al., 2019). Interestingly, the report showed a concentration dependent alteration of miRNA in PTSD group (Lee et al., 2019). The concentration changes of two miRNAs from EV (miR-203a-3p) and EVD plasma (miR-339-5p) were validated in an independent cohort of 20 Veterans (Lee et al., 2019). This may suggest EV as possible biomarkers to identify PTSD in Veterans. More studies are warranted to validate the finding in diverse cohorts. The observation may highlight the benefits of EV as a treatment module.

In summary, a growing literature has started to include miRNA in the study of PTSD among Veterans. The extant literature on miRNA and PTSD shows several miRNAs that are dysregulated in Veterans with PTSD compared to no PTSD control groups. The most common medium for sample utilization has been PMBCs, with other samples using serum or plasma. Though limited by small samples in many studies, data showed a spectrum of altered miRNA, consistent with system dysregulation seen in PTSD. Further, miRNA linked to immune function and inflammation were consistently associated with PTSD. However, the studies reviewed varied on several domains, including the use Veterans of different eras, inclusion of combat related and non-combat related PTSD as well as the use of control groups (i.e., some used trauma exposed Veterans while others used civilian

healthy volunteers). These differences, as well as examination of different miRNAs limit conclusions that can be drawn. However, there are several directions for future research.

## Future Perspectives of miRNAs in PTSD

In this review article, we have discussed and presented evidence that miRNAs hold a key role in PTSD among Veterans. There is a prospect in future of using miRNAs as circulatory biomarker in detection of PTSD. Below we discuss directions for future research, particularly as it relates to epigenetic modification and the HPA axis, as well as treatment implications.

## Epigenetic modification in PTSD

Recent research has indicated an epigenetic modification in the central nervous system that may influence alterations in neurological diseases (Provencal and Binder, 2015). The term 'epigenetics' signifies chemical modifications to the chromatin structure that alter gene transcription while the DNA sequence remains un-altered. The alteration includes DNA methylation, DNA hydroxy-methylation, histone modifications and the processes are designated as methylation, acetylation, and phosphorylation, respectively. Other epigenetic modulators are non-coding RNAs like miRNAs which act as translational repressors (Auger and Auger, 2013; Bam et al., 2016, Roth, 2014; Martin et al., 2018). The war Veterans are no exception in this epigenetic modification. There were reports that DNA methylation contributed significant role in the pathophysiology of PTSD as the process is essentially connected with gene regulation (Mehta et al., 2017; Uddin M et al., 2010; Rusiecki et al., 2012). Using a sample of OEF/OIF Veterans, Rusiecki *J et al* (Rusiecki et al., 2012) showed two repetitive elements, the long-interspersed nucleotide element 1 (LINE-1) and the interspersed Alu were hypomethylated in post-deployment situation. The authors suggested their findings as highlighting potential resilience or vulnerability factors (Rusiecki et al., 2012). The study reviewed above using Gulf War Veterans showed a link between Wnt signaling pathway and miR-7113-5p in PBMCs of PTSD subjects, which suggests both miRNA dysregulation and histone modifications (Bam et al., 2020). The miRNA modulation in epigenetic modification is intriguing as miRNA can regulate a set of gene expression at post-transcriptional level.

## miRNA-FKBP5-HPA axis-PTSD

A dysfunctional HPA-axis is a hallmark in PTSD (Speer et al., 2019). Further, recent research has posited that unique aspects of Veterans' history, their deployment characteristics and their readjustment to civilian life may uniquely impact HPA axis functioning in ways that make them more vulnerable to maladaptive coping, such as alcohol use (Szabo Y et al., 2020). The HPA-axis is designed to respond to stress. Physiological stimuli and stress activate it, ultimately leading to the release of cortisol from the adrenal cortex (Olf et al., 2006; Yehuda, 2009; Carrasco and Van de Kar, 2003). Essentially, corticotropin-releasing factor is released from the hypothalamus and stimulates the synthesis and release of ACTH from the pituitary. The ACTH binds to receptors in the adrenal cortex and promotes the release of glucocorticoids (GCs) from the adrenal cortex (de

Kloet, 2003). The circulating GCs, including cortisol, are counter regulated by negative feedback mechanism within the HPA and is critical for stress response maintenance. GCs mediate their effect through glucocorticoid receptor (GR) and mineralocorticoid receptors (MR) (Castro-Vale, 2016). FK506-binding protein 5 (FKBP5), a co-chaperon, is a critical modulator in GR signaling and has been implicated in the development of PTSD (Fries, Gassen and Rein, 2017; Hawn et al., 2019). Previous research has shown an association between FKBP5 and the development of PTSD including studies of Veterans (Binder et al., 2008; Fani et al., 2016; Young et al., 2018). As miRNA emerges as a circulating biomarker in PTSD, it would be interesting to assess the role of miRNA in the regulation of FKBP5. Using both *Fkbp5* knock-out (KO) mouse model and human PTSD subjects, a panel of miRNA was identified which correlated with serum marker and miRNA expression in the pathology of PTSD at molecular and behavioral levels (Kang et al., 2020). The candidate miRNA derived from mouse study was validated with human subjects with PTSD and showed exosomal FKBP-linked miRNA in the blood as a possible biomarker. The study further determined the neuronal correlate with serum biomarker depicting HPA-axis and miRNA expression, with a composite score of miRNA expression positively correlated with higher prefrontal/limbic cerebral blood flow and a higher grey matter volume ratio within the PTSD group (Kang et al., 2020). This is the first study to show a panel of differential miRNAs profiling in *Fkbp5* KO mice, a critical modulator in HPA-axis. Furthermore, a follow-up study by the same group conducted RNA sequence analysis using WT (wild type) and *Fkbp5* KO mice with restrain stress, a form of physical and mental stress that is induced by placing the mice in a plastic tube in order to block their movements, to determine the specific miRNA affected in medial prefrontal cortex (mPFC). The study showed that 41 miRNAs were dysregulated, of which, 23 miRNAs were reduced and, 18 miRNAs were increased. Among upregulated miRNA, miR-690 showed significantly high level of expression and was chosen for further characterization (Park et al., 2020). Using green fluorescent protein (GFP)-tagged recombinant adeno-associated virus (rAAV) and viral construct containing miR-690 (rAAV-GFP-miR-690) into the pre-limbic cortices of the mPFC of mice, the authors showed in restrain stress mouse model that overexpression of miR-690 revealed higher sucrose preference and lower immobility time compared to stressed mice (Park et al., 2020). This finding may provide novel insights into the epigenetic regulation of stress-associated biological functions like PTSD. Further research in human models and specifically Veterans are needed to verify these findings. However, identification of miRNA in FKBP5 modulation in PTSD may uncover new mechanism of PTSD development and offer possible therapeutic target.

### Other directions for future research

One potential avenue for future research is focusing on enzymes that play a role in generating miRNA. One study showing promising results is one that focused on *Dicer1*, an enzyme that generates mature miRNAs, which regulate gene expression. In this study, levels of *Dicer1* were associated with increased amygdala activation to fearful stimuli, a neural correlate for PTSD among civilians (Wingo et al., 2015). The finding specifically demonstrated that miR-3130-5p was significantly reduced in PTSD with depression subjects compared to controls with a history of trauma but no PTSD or depression, indicating that



*DICER1* and miR-3130-5p impart a critical role in the pathogenesis of PTSD (Wingo et al., 2015). This is the first human study showed *DICER1* and miRNA modulation in underpinning the PTSD comorbid with depression. Given that PTSD and depression are highly comorbid in Veterans (Ikin et al., 2010) and the prevalence of both disorders is associated with greater psychological burden than PTSD alone (Nichter et al., 2019), investigation into their comorbidity may provide important information for etiology of these disorders and offer new avenues for treatment.

The studies included in this review used syndromal PTSD versus no PTSD controls. Within the control groups, they ranged from Veterans with combat exposure to healthy civilian volunteers. Future research using trauma-exposed Veterans will help inform the specificity of PTSD for changes in miRNA regulation. Finally, individuals can have significant symptoms, without meeting criteria for the PTSD syndrome. Future studies are needed to understand how the presence of symptoms compared to the severity of symptoms associated with the alteration of miRNAs. Some preliminary research with a civilian sample has found associations between ratios of miRNA expression and PTSD symptom severity (Kang et al., 2020).

## Treatment Implications

At present, it may be premature to offer miRNA as psychiatric therapeutic tool but, possibilities exist. Introducing targeted miRNA into the central nervous system would be challenging and several off-target effects would have dire side effects. However, current knowledge in bioinformatics has provided powerful information regarding precise targets among multiple predicted targets. Moreover, identification and validation of miRNA and its target gene(s) would further enrich our understanding of underlying molecular mechanism of PTSD. The novel RNA-based therapeutics can be developed by taking the advantage of CRISPR/CAS9 gene editing (Dominguez et al., 2016). Furthermore, the FDA approved selective serotonin reuptake inhibitors (SSRIs) currently used for PTSD treatment may be considered for miRNA modulation. Regarding miRNA involved in SSRI, mouse model of PTSD showed that fluoxetine is associated with a significant reduction in miR-1971 expression (Schmidt et al., 2013). However, the use of SSRIs to treat PTSD in Veterans has been mixed. PTSD participants treated with SSRI (antidepressants) showed modest protective effect against relapse relative to placebo subjects (Martenyi et al., 2002; Martenyi and Soldatenkova, 2006; Cavaljuga et al., 2003). However, other studies have shown fluoxetine was not superior to placebo in a study of combat Veterans (Hertzberg et al., 2000). Furthermore, a study conducted by Copeland L *et al* showed there some SSRIs are associated with increased risk of long QT syndrome, a disorder of the heart's electrical system and, there was no significant risk using two SSRI drugs, citalopram and fluoxetine, in PTSD (Stock et al., 2018). Together, these suggest that fluoxetine may have less QT prolongation risk and, may cause change in miRNA. More research is needed to observe the dynamic of circulatory miRNAs in SSRI treated patients to understand factors associated with efficacy in Veterans, as it may that there is subset of Veterans who may benefit from this treatment most. Further, venlafaxine has showed increased remission rates compared to other SSRIs in a routine clinical care study of Veterans enrolled in VA care (Shiner et

al., 2010). Thus, future research examining whether venlafaxine or other SSRI agents alter miRNA may inform better treatment efficacy in Veterans.

The authors are not aware of any research suggesting psychotherapy has been associated with changes in miRNA. However, other research in DNA methylation, another epigenetic modification, in psychotherapy treatment is emerging (Wolf et al., 2019). There is research showing that pre-treatment methylation has prognostic value in that it predicts outcomes (Yehuda et al., 2013). If targeting miRNA can impact methylation, then Veterans who previously didn't respond to psychotherapy might become responders and, thus, leading to better treatment responses. Together, studies correlating miRNA alteration with clinical outcomes may contribute in the development of biomarkers and miRNA-based therapeutics in PTSD diagnosis and prognosis.

## Conclusion

Veterans are suffering from many psychiatric disorders due to exposure of concussive brain injuries resulted in TBI and large quantities of psychological trauma exposure that result in high rates of PTSD. There is a great prospect of use of miRNA as biomarkers for the diagnosis, prognosis and therapeutic opportunities for Veterans with psychiatric disorders, including PTSD. The fact that differential expression of circulatory miRNA originated from neuronal dysfunction in brain tissue, miRNA have been associated with several disease processes pertaining to brain tissues advocating the potential use of miRNA as a next generation biomarker for the treatment of neuropsychiatric conditions. Circulating miRNAs in Veterans with PTSD can be used as independent biological indexes. The insights may offer gene networks related PTSD symptomatology, improve biological mechanisms of PTSD and provide pharmacological targets avenue. The implication and ramification of miRNA in psychiatric research is at budding stage compared to the more established study in cardiovascular or cancer fields; therefore, larger studies are warranted using Veterans with appropriate control cohort. The present review summarizes the small literature on miRNA in Veterans, considers directions for future research and proposes how this field of study can be used to improve the treatment of PTSD for Veterans. The outcome will help us to understand the deeper function and, novel insight into the mechanism of miRNA and, the target genes in the pathophysiology of PTSD in war Veterans. Finally, it may lead to the clinical application of miRNAs in PTSD diagnosis and prognosis.

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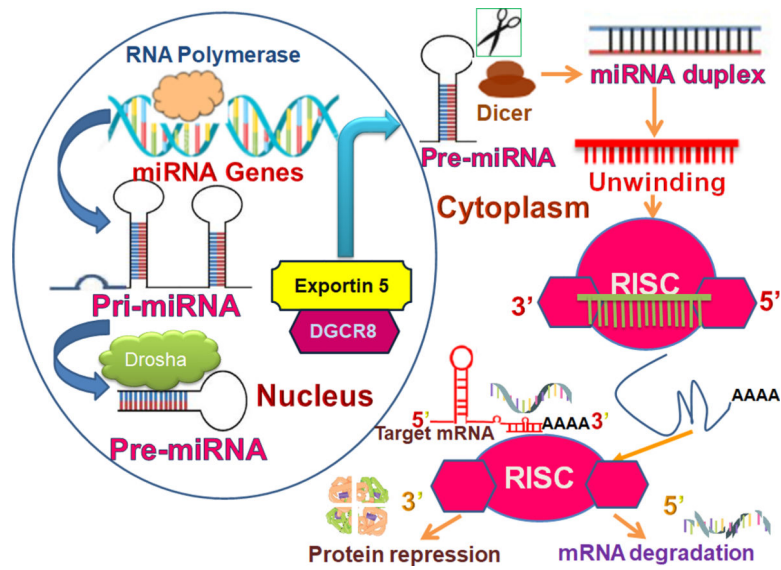
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**Figure 1.** Schematic of miRNA biogenesis. Pri-miRNA, Primary miRNA; Pre-miRNA, Precursor miRNA; RISC, RNA-induced silencing complex; AGO, Argonaute protein; DGCR8, DiGeorge syndrome critical region 8