Chapter 8

microRNAs As Novel Players in Depression Pathogenesis and in the Mechanisms of Action of Fluoxetine and Other Antidepressants

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Abstract

Major depressive disorder (MDD) is a major public health concern. Although much work has been done to characterize MDD, a large number of MDD patients do not respond to the currently available medications and the relapse rate for depression is quite high. Thus, there is an urgent need to fully understand the neurobiological abnormalities associated with MDD and develop target-based therapeutic approaches. In this context, microRNAs (miRNAs) have emerged as important gene regulators which are involved in many higher brain functions. Because miRNAs show a highly regulated expression, they contribute in the development and maintenance of a specific transcriptome and thus have the unique ability to influence a wide range of physiological and disease phenotypes. Recent studies demonstrating involvement of miRNAs in several aspects of neural plasticity, stress response, and more direct studies in human postmortem brain, peripheral blood cells - provide strong evidence that miRNAs can not only play a critical role in MDD pathogenesis but can also open novel avenues for the development of therapeutic targets. In this chapter, these aspects are discussed in a comprehensive manner.

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## Introduction

Major depressive disorder (MDD) is the most prevalent of psychiatric disorders. It affects about 150 million people worldwide at any point in time (Wang et al., 2007) and about 17% of the US population during their lifetime (Kessler 2005). MDD is associated with poor quality of life and premature death, as over 60% of suicides are related to MDD. Although much work has been done to characterize MDD, about 40% of MDD patients do not respond to the currently available medications (Fava et al., 1996). This is partially a result of poor understanding of the molecular pathophysiology underlying MDD.

miRNAs, a class of small non-coding RNAs, are the major regulators of neural plasticity and higher brain functioning (Leistedt et al., 2013; Ota et al., 2013). By regulating gene expression in a highly a coordinated and cohesive fashion (Malphettes, 2006), miRNAs can participate in the development and maintenance of the transcriptome and thereby can influence physiological and disease phenotypes. The roles of miRNAs in various neuropsychiatric diseases are being vigorously investigated as miRNAs can not only play a direct role in disease pathogenesis, but they can also help in identifying the nature of disordered pathways implicated in such pathogenesis (Choi et al., 2005; Mo et al., 2009; Xu et al., 2011; Stäehler et al., 2012). This is also crucial in the development of novel therapeutic drugs.

Although studies examining the role of miRNAs in MDD are in early stages, several studies from human postmortem brain and animal model systems have provided evidence suggesting that miRNAs may be crucial in the etiopathogenesis of MDD. Also, there are multitudes of studies which points to the involvement of miRNAs in synaptic plasticity and neurogenesis; crucial factors in MDD pathophysiology. The aim of this chapter is to review the current status of our understanding of the role of miRNAs in MDD. We have focused on studies showing involvement of miRNAs in neuroplasticity, neurogenesis, and in stress-related behavioral response. We have also summarized findings from human postmortem brain and blood cell studies of MDD patients as well as the role of miRNAs in the mechanism of action of antidepressants.

### miRNAs: Synthesis and Mechanisms of Post-Transcriptional Repression

miRNAs belong to the eukaryotic family of small non-coding RNAs comprised of more than 200 family members per species and building a largest family accounting for 1% of the genome (Kim, 2005). Recently released 20th version of the mirBase database shows 24521 entries representing hairpin precursor miRNAs expressing 30424 mature miRNA products in 206 species (www.miRBase.org). In humans, so far, 2578 mature miRNAs and 1872 precursor miRNAs have been annotated (Kozomara and Griffiths-Jones, 2014).
miRNAs are single stranded molecules with mature length of 20-22 nucleotides that control the activity of about 50% of protein-coding genes (Friedman and Jones 2009). These miRNAs are transcribed by RNA polymerase II or III as a primary transcript followed by processing with a set of enzymes belonging to the RNase III family, giving rise to an effector molecule with impact on post-transcriptional gene regulation by either directly mediating mRNA decay or repressing translation with a precise mechanism (Bartel, 2004).

Conversion of Primary miRNA to Precursor miRNA: Single stranded mature miRNAs are derived from longer double stranded molecules with atypical hairpin stem-loop structure known as primary miRNA (pri-miRNA). Generally, both transcriptional and post-transcriptional regulation act upon miRNA biogenesis pathway to produce the mature transcript, (Ameres & Zamore, 2013). However, a few of them use a non-canonical pathway to become mature. They are mainly derived from intronic regions of protein-coding genes without recruiting splicing machinery for their biogenesis and popularly known as ‘Mirtron’ (Westholm et al., 2012). After transcription by RNA polymerase II (Lee et al., 2004), pri-miR is converted to precursor miRNA (pre-miR) by the catabolic activity of RNase III family member Drosha. Multiple forms of ~60 base long hairpin structure of precursor miRNA has been reported to be produced from a single pri-miRNA (Gregory et al., 2004). In mammalian cells, Drosha forms a microprocessor complex with another protein named Digeorge Syndrome at Critical Region 8 (DGCR8) (Han et al., 2006). This complex structure helps increase activity of Drosha by modulating its affinity for the substrate and by increasing cleavage site accuracy (Ameres & Zamore, 2013).

After Drosha mediated processing in nucleus, pre-miRNAs are transported to cytosol. This transportation is mediated by the nuclear transporter protein Exportin 5 and energized by Ran-GTP complex (Lund et al., 2004). Once pre-miRNAs reach cytoplasm, another RNase III enzyme Dicer cleaves pre-miRNA into a shorter form, producing single stranded miRNAs with a length of ~22 nucleotides (Zhang et al., 2002). However, this is not a unifying feature in miRNA biogenesis. In certain cases, such as in zebra fish, pre-miR -451 is processed without being acted on by Dicer enzyme (Yang et al., 2010). Due to its small size, precursor miRNA-451 is directly recruited on the silencing complex for further action (Cheloufi et al., 2010).

Mechanisms of Gene Silencing by miRNA: One of the miRNA/miRNA* duplexes is loaded onto an Argonaute homologue protein (Ago, isoforms of eIF2c) to generate the effector complex, known as RNA-induced silencing complex (RISC). The other miRNA* strand is degraded. RISC complex assembly is mainly comprised of Dicer, double stranded RNA binding domain protein TRBP (TAR-binding protein), PACT (Protein Activator of PKR) and Argonaut 2 (Ago2). In this complex the requirement of TRBP and PACT are not indispensable for Dicer activity for pre-miRNA processing but they do intensify the functionality of Dicer (Chendrimada et al., 2005; Lee et al., 2006).

RISC binds to specific “short-seed” sequences located predominantly within the 3’ untranslated region (3’ UTR) of target mRNAs and either interferes with translation of the mRNA or reduces mRNA levels by degradation. miRNA-mediated translational inhibition depends upon the 5’ cap region of the target mRNA. Ago proteins can stimulate miRNA-dependent inhibition of translation by competing with eIF4E for the 5’cap binding site, thus preventing circularization of mRNA and lowering initiation efficiency (Mathonnet et al., 2007). Because RISC/miRNA complex recognizes target mRNA based on a seed region containing 2-8 nucleotides at the 5’end of miRNA, it provides a mechanism by which one
miRNA can target several mRNAs (Brodersen et al., 2009). RISC can also associate with 60S ribosome and elf6 (Chendrimada et al., 2007). elf6 regulates the formation of translationally active 80S subunit; so by regulating association with elf6, miRNAs can modify polysome formation and expose target mRNAs for degradation (Chendrimada et al., 2007). MiRNA-mediated regulation of mRNA stability is another mechanism by which miRNAs suppress expression of specific mRNA. Using miR-125b and let-7 as representative miRNAs, Wu et al. (2006) showed that in mammalian cells, miRNA initiates the reduction in mRNA abundance through accelerated deadenylation, which leads to rapid mRNA decay.

Besides regulating translational and mRNA decay processes, it has been shown that miRNA can also regulate gene transcription by targeting transcription factors. In this case, levels of transcription factors are downregulated by miRNAs, which in turn cause less expression of mRNA, leading to reduced protein synthesis (Kosik, 2006; Michalak, 2006)

**Role of miRNAs in MDD Pathogenesis**

Multiple lines of investigation suggest that MDD is associated with altered neuroplasticity (Leistedt and Linkowski, 2013; Ota and Duman, 2013). In addition, neurogenesis plays a crucial role in depression and in the mechanism of action of antidepressants. Specifically, we and other investigators have shown that transactivation of transcription factor CREB is altered (Dowlatchahi et al., 1998; Dwivedi et al., 2003), along with expression of neuroplasticity genes such BDNF, NGF, NT-3 and their cognate TRK receptors in depressed brain (Dwivedi et al., 2003, 2009a). Hypoactivation of genes involved in ERK2 and PI3 kinase pathways, such as ERK1/2, MAP kinase kinase1, ERK5, Rap-1, B-Raf, and Epac (Dwivedi et al., 2001; 2006a; 2006b; 2007; Gourley et al., 2008;; Qi et al., 2008; Dwivedi et al., 2009; Todorovic, Dwivedi et al., 2009b; Yuan et al., 2010) has also been found in postmortem brain of depressed subjects. In addition, the expression of protein kinase A and protein kinase C, which are integral parts of the adenylyl cyclase-cyclic adenosine monophosphate and phosphoinositide signaling systems and which are regulators of neural plasticity, are down regulated in depressed suicide subjects (Pandey et al., 1997; Dwivedi and Pandey, 2008).

Interestingly, miRNAs play a critical role in both neurogenesis and neuroplasticity either by directly impeding the translation process of target mRNAs with a post-transcriptional gene silencing mechanism or indirectly by targeting transcription factors. In the following sections we describe the role of miRNAs in neurogenesis and neural plasticity. In addition, miRNA modulations in brain and blood cells of MDD patients have been discussed in greater detail.

**Regulation of Neurogenesis by miRNAs:** Several studies indicate that MDD is associated with decreased hippocampal neurogenesis and that mechanisms of action of antidepressants are associated with increased neurogenesis. Interestingly, ~20% to 40% of miRNAs in the brain are developmentally regulated. miRNAs are not only important during embryonic development but they are also critical in regulating adult neurogenesis (Miska et al., 2004; Sempere et al., 2004). One of the most important miRNAs that regulate neurogenesis is miR-124. miR-124a is the most abundant miRNAs in the mammalian brain and accounts for 25-48% of all brain-expressed miRNAs (Lagos-Quintana et al. 2002). Transfection of mouse neuronal stem cells with miR-124a stimulates neuron-like
differentiation by promoting neuron-specific class III beta-tubulin 1 and MAP2 expression, causing G0/G1 cell cycle arrest (Silber et al. 2008).

In addition, knockdown of this miRNA maintains subventricular zone stem cells as dividing precursors, whereas its ectopic expression leads to precocious and increased neuron formation in mice (Cheng et al. in 2009). The primary target of miR-124 is SRY-box transcription factor Sox9. miR-124-mediated overexpression of Sox9 abolishes neuronal differentiation, whereas Sox9 knockdown leads to increased neuron formation (Chen et al. 2009). Recently, Szulwach et al. (2010) showed that MeCP2, a DNA methyl-CpG-binding protein, can epigenetically regulate miR-137, which is regulated by Sox2, a core transcription factor in stem cells. These investigators showed that overexpression of miR-137 promoted the proliferation of adult neural stem cells, whereas a reduction of miR-137 enhanced adult neural stem cell differentiation. In addition, miR-137 post-transcriptionally repressed the expression of Ezh2, a histone methyltransferase and polycomb group protein. The miR-137-mediated repression of Ezh2 resulted in a global decrease in histone (H3) trimethylation at lysine 27 residue. Coexpression of Ezh2 rescued phenotypes associated with miR-137 overexpression, thus demonstrating a fascinating cross-talk between miRNA and epigenetic regulation in the modulation of adult neurogenesis with a feedback and feed forward loop model (Szulwach et al., 2010).

**Regulation of Neural Plasticity by miRNAs:** A growing body of evidence indicates that a specific population of miRNAs is expressed within dendrites and in dendritic spines, where they contribute in the regulation of local protein synthesis, and thus dendritic spine morphogenesis. In fact, Lugli et al. (2008) have demonstrated that the entire synthesis machinery for miRNAs is localized at the synapse in mouse forebrain. Similarly, Kye et al. (2007) showed that most neuronal miRNAs are detectable in the dendrites. Amongst them, the most striking miRNA is miR-26a, which targets microtubule-associated protein 2, a protein involved in neural reorganization (Kye et al., 2007). Earlier, Schratt et al. (2006) showed that expression of Limk1, a protein that controls dendritic spine development, is regulated by miR-134 in the synaptodendritic compartment. They also found that exposure of the hippocampal neurons to BDNF relieves miR-134 inhibition of Limk1 translation, causing an increase in the size of dendritic spines. Siegal et al. (2009) showed that miR-138, which regulates the expression of Acyl protein thioesterase 1 (APT1), is highly localized within dendrites and negatively regulates the size of dendritic spines in rat hippocampal neurons. They found that palmitoylation of Ga13 by APT1 is critical in dendritic spine morphogenesis.

BDNF, a critical player in neural plasticity has been shown to regulate miR-132 (Kawashima et al., 2011). MiR-132 induces neurite outgrowth and modulates dendritic morphology of cortical and hippocampal neurons by repressing p250GAP (Vo et al. 2005; Wayman et al., 2008). Deletion of miR-132 leads to a dramatic decrease in dendrite length, arborization, and spine density (Magill et al., 2010). MiR-132 expression has also been shown to promote neuronal maturation and synapse formation by regulating MeCP2 expression (Fukada et al., 2005; Jugloff et al., 2005; Klien et al., 2007). Interestingly, MeCP2 controls BDNF expression, which itself leads to induction of miR-212/132 expression. With this, miR-132 can take part in a feedback mechanism involved in the homeostatic control of MeCP2 expression (Nudelman et al., 2010). Besides, CREB, which modulates the transcription of several genes with cAMP responsive elements in their promoter regions (Silva et al., 1998; Benito and Barco 2010; Sakamoto et al. 2011), also targets miR-132. In addition, miR-212 is
also targeted by CREB and plays a role in neuronal development and function (Vo et al., 2005; Impey et al., 2004).

**miRNAs and Stress Response:** Stress, a major factor in MDD, differentially regulates miRNA expression in hippocampus, amygdala (Mcearson et al., 2010) and frontal cortex (Rinaldi et al., 2010), key brain regions involved in emotion and cognitive processes. Early life stress, another critical factor in development of MDD (Raabe, 2013), has also been shown to impact miRNAs in mice prefrontal cortex (Uchida et al., 2010). Genetic differences in miRNA expression can influence an individual’s coping response to a stressor. In this regard, it has been shown that stress-sensitive F344 rats, which release excessive corticosterone (CORT) in response to a stressor (Uchida et al., 2010), express higher hypothalamic miR-18a. Mechanistically, miR-18 binds to 3’UTR of glucocorticoid receptor and reduces its expression (Vreugdenhi, 2009). This results in reduced negative feedback leading to increased CORT release. The importance of this increased release was demonstrated by exposing of neurons to excess CORT, which results in decreased BDNF-dependent neuronal functions via suppression of miR-132 expression (Kawashima et al., 2010).

Recently we studied the expression of a large set of miRNA in frontal cortex of rats who developed behavior (learned helpless [LH]) that resembles stress-induced depression vs. those that did not develop depression-like symptoms (non-learned helpless [NLH]) despite similar exposure to inescapable shock (Smalheiser et al., 2011). We found that NLH rats show a robust adaptive miRNA response to inescapable shocks whereas LH rats show a markedly blunted miRNA response. An impressive number of miRNAs exhibited down regulated expression in NLH rats when compared with control group. These include: miR-96, miR-141, miR-182, miR-183, miR-183*, miR-198, miR-200a, miR-200a*, miR-200b, miR-200b*, miR-200c, and miR-429. These miRNAs were encoded at a few shared polycistronic loci, suggesting that their down regulation was coordinately controlled at the level of transcription. Creb1 mRNA was identified as one of potential targets of several miRNAs and reciprocally Creb1 was found to be a trans-activating factor on the upstream region of miR-96, miR-182, miR-183, miR-200a, miR-200b, miR-200c, miR-220a*, and miR-200b.*

**miRNA Modulation in MDD Postmortem Brain:** We are the first to study miRNAs in the human postmortem brain of subjects who had major depression and died by suicide (Smalheiser et al., 2013, 2014). We found that 21 miRNA were significantly downregulated in the prefrontal cortex of MDD subjects-(listed in Table 1). When analyzed individually, almost half of the down-regulated miRNAs were encoded at chromosomal loci near another miRNA and are possibly transcribed by the same pri-miRNA gene transcripts (miR-142-5p and 142-3p; miR-494, 376a*, 496, and 369-3p; miR-23b, 27b and 24-1*; miR-34b* and 34c; miR-17* and 20a). In addition, three pairs of miRNAs were encoded at distances greater than 100 kb but still found to lie within the same chromosomal region (miR-424 and 20b at Xq26.2-3, 377 kb apart; miR-142 and 301a at 17q22, 820 kb apart; miR-324-5p and 497 at 17p13.1, 205 kb apart). This suggests that at least some of the down-regulated miRNA expression is due to decreased transcription. Many of the down-regulated miRNAs also shared 5’-seed sequences that are involved in target recognition. For example, identical seed sequences are shared by a) miR-20a and 20b; b) miR-301a and 130a; and c) miR-424 and 497. Additionally, a 6-mer nucleotide motif is shared by miR-34a, 34b* and 34c, and strikingly, a 5-mer motif (AGUGC) within the 5’-seed is shared by 5 of the affected miRNAs (miR-148b, 301a, 130a, 20a, 20b) that is predicted to bind Alu sequences within the 3’-UTR
region of target mRNAs. This suggests that the down-regulated miRNAs should exhibit extensive overlap among their mRNA targets.

When pair-wise correlations were made (a complementary method of analyzing the miRNA expression data to identify pairs of miRNAs that are co-regulated in their expression, up or down, across individuals within a single group), a set of 29 miRNAs were identified, none of which were pair-wise correlated in the normal control group, but which formed a very extensive inter-connected network in the depressed group. Several of the miRNAs (let-7b, miR-132, 181b, 338-3p, 486-5p, and 650) were “hubs” correlated with four to nine other miRNAs in the network. Target analysis revealed that many of the targets are transcription factors, and nuclear, transmembrane and signaling proteins. Intriguingly, 4 different down-regulated miRNAs target VEGFA (miR-20b, 20a, 34a, 34b*), a molecule implicated in depression in both humans and in animal models. Other validated targets include BCL2 (miR-34a), DNMT3B (miR-148b), and MYCN (miR-101, 34a). Among predicted targets, estrogen receptor alpha, ESR1, was predicted to be targeted by 3 different down-regulated miRNAs (miR-148b, 301a, 496). Others targeted by 3 or more affected miRNAs include ubiquitin ligases (UBE2D1 and UBE2W), signal transduction mediators (CAMK2G, AKAP1), the splicing factor NOVA1 that regulates brain-specific alternative splicing; the GABA-A receptor sub-unit GABRA4; calcium channel CACNA1C; and brain-active transcription factors including SMAD5, MITF, BACH2, MYCN, and ARID4A. Several of these predicted targets interact with validated targets in MDD; for example, ARIA4A binds E2F1; SMAD5 binds RUNX1; and estradiol treatment decreases E2F1 levels in prefrontal cortex (Wang et al., 2004). BACH2 transcription factor binding sites have been identified upstream of many brain-expressed miRNAs (Wu and Xie, 2006). Retinoblastoma binding protein 1 (ARIA4A) is of interest because it recruits histone deacetylases and regulates gene expression via chromatin-based silencing.

Selected target proteins such as DMNT3b, VEGFA, and BCL2 were studied by examining their expression in depressed suicide brain. DMNT3b was strongly up-regulated in the depressed suicide group, whereas BCL2 was downregulated. Several miRNAs that were co-regulated with their targets showed a strong positive correlation with DMNT3b and BCL2. A variety of factors such as transcription factor activity and turnover rate, as well as possible regulatory effects of other miRNAs may also be responsible for changes in mean expression levels of these target proteins. In addition, DMNT3b levels showed an extremely strong positive correlation with miR-148b across subjects (r = 0.91 in controls, r = 0.94 in the depressed suicide group). Similarly, BCL2 was strongly and positively correlated with miR-34a (r = 0.92 in healthy controls, r = 0.82 in the depressed suicide group). The correlation of miR-34a was positive in healthy controls, but inverse in the depressed suicide group, presumably reflecting a reorganization of miRNA-target networks (Smalheiser et al., 2012).

Previous studies indicate that TrkB.T1, a BDNF receptor lacking a tyrosine kinase domain that is highly expressed in astrocytes and regulates BDNF-evoked calcium transients, is downregulated in frontal cortex of suicide subjects (Ernst et al., 2009). In a recent study, Maussion et al. (2012) examined whether this TrkB.T1 gene is regulated by miRNAs. The investigators found that Hsa-miR-185* and Hsa-miR-491-3p were upregulated in suicide completers with low expression of TrkB.T1; FDR.1. Bioinformatic analyses revealed five putative binding sites for the DiGeorge syndrome linked miRNA Hsa-miR-185* in the 3'UTR of TrkB.T1, but none for Hsa-miR-491-3P. The increase of Hsa-miR-185* in frontal cortex of
suicide completers was validated then confirmed in a larger, randomly selected group of suicide completers, where an inverse correlation between Hsa-miR-185* and TrkB.T1 expression was observed. Silencing and overexpression studies performed in human cell lines confirmed the inverse relationship between hsa-mir-185* and trkB-T1 expression. Furthermore, luciferase assays demonstrated that Hsa-miR-185* binds to sequences in the 3'UTR of TrkB.T1. These results suggest that an increase of Hsa-miR-185* expression levels regulates, at least in part, the TrkB.T1 decrease observed in the frontal cortex of suicide completers and further implicate the 22q11 region in psychopathology.

Alterations in metabolic enzymes of the polyamine system have been reported to play a role in predisposition to suicidal behavior (Fiori et al., 2011). Recently, Lopez et al. (2013) examined whether dysregulation of polyamine genes in depressed suicide completers could be influenced by miRNA-mediated post-transcriptional regulation. These investigators identified several miRNAs that target the 3'UTR of polyamine genes SAT1 and SMOX. When the expression of 10 miRNAs in the prefrontal cortex of suicide completers and controls using qRT-PCR were profiled, they found that several miRNAs showed significant up-regulation in the prefrontal cortex of suicide completers compared to psychiatrically-healthy controls (miR-124, miR-139-5p, miR-195, miR-198, miR-320c, miR-33b, miR-34a, miR-34c-5p, miR-497, miR-873). However, they found that only miR-139-5p and miR-320c were inversely correlated with polyamine gene SAT1 whereas miR34c-5p and miR-320c were inversely correlated with polyamine gene SMOX. These results suggest a relationship between miRNAs and polyamine gene expression in the suicide brain, and postulate a mechanism for SAT1 and SMOX down-regulation by post-transcriptional activity of miRNAs.

Polymorphism in genes responsible for microRNA processing has also been documented to play a role in depressive disorder. For example, miRNA processing genes DGCR8, Ago1, and GEMIN4 were found to harbor polymorphism and exhibited a strong correlation with pathophysiology of depression (He et al., 2012). Variant allele of DGCR8 rs3757 was associated with increased risk of suicidal tendency and improved response to antidepressant treatment, whereas the variant of AGO1 rs636832 showed decreased risk of suicidal tendency, suicidal behavior, and recurrence (He et al., 2012). This information could be very useful and can be used as a diagnostic tool for early detection of depression pathophysiology.

MiRNA Modulation and Antidepressant Response

**MDD Patient Population Studies**

Recently, several studies have indicated that mechanisms of action of antidepressants could be associated with modulation in expression of miRNAs. Belzeaux et al. (2012) profiled miRNAs in peripheral blood mononuclear cells collected from 16 severe MDD patients and 13 matched controls at baseline, at 2 and 8 weeks after antidepressant treatment. Comparison of miRNA expression between MDD patients and controls at baseline and at 8 weeks showed a similar number of dysregulated miRNAs (14 miRNAs, with 9 miRNAs upregulated and 5 downregulated, Table 1). Two miRNAs showed stable overexpression in MDD patients during the 8-week follow-up compared with controls (miR-941 and miR-589). They also identified miRNAs exhibiting significant variations of expression among patients with clinical improvement (7 upregulated and 1 downregulated). Fourteen dysregulated
miRNAs had putative mRNA targets that were differentially expressed in MDD, suggesting that a common RNA regulatory network functions in MDD. These results suggest the potential utility of miRNA signatures as markers of MDD evolution.

In a whole miRnome analysis, Bocchio-Chiavetto et al. (2013) examined blood miRNAs from 10 MDD subjects after 12 weeks of treatment with escitalopram. They found that 30 miRNAs were differentially expressed after the escitalopram treatment: 28 miRNAs were up-regulated, and 2 miRNAs were down-regulated. Thirteen of them (let-7d, let-7e, miR-26a, miR-26b, miR-34c-5p, miR-103, miR-128, miR-132, miR-183, miR-192, miR-335, miR-494 and miR-22) play a role in the neural plasticity and stress response and in the pathogenetic mechanisms of several neuropsychiatric diseases. As mentioned previously, miR-132 exerts critical functions in the biological circuits implicated in neurogenesis and synaptic plasticity, stimulating axonal and dendritic outgrowth in different brain areas (Mellios, 2011). This miRNA, together with miR-26a, miR-26b and miR-183, widely contributes to the action of the neurotrophin BDNF in the brain (Wayman, 2008; Rinaldi et al., 2010; Caputo et al., 2011; Kawashima et al., 2010). miR-132, miR-26a, miR-26b, miR-183, let-7d, let-7e, miR-26b, miR-103, miR-128, miR-494 and miR-22 (Dwivedi et al., 2011; Serafini et al., 2012) play a role in the pathogenesis of psychiatric disorders and in the mechanism of action of antipsychotic drugs and mood stabilizers.

To differentiate responders vs. non-responders, Oved et al. (2012) examined the growth inhibition response of SSRI paroxetine in lymphoblastoid cell lines obtained from healthy female individuals. Out of 80, they selected 8 LCLs which exhibited high or low sensitivities to paroxetine. These LCLs were chosen for miRNA profiling. They found that miR-151-3p had 6.7-fold higher basal expression in paroxetine-sensitive LCLs. miRNAs miR-212, miR-132, miR-30b*, let-7b and let-7c also differed by >1.5-fold between the two LCL groups. These findings suggested that profiling these specific miRNAs can differentiate responders vs. non-responders specifically for paroxetine treatment.

Animal Model Studies

Response to ECT, fluoxetine and ketamine: In rat model of electroconvulsive therapy (ECT) Ryan et al. (2013) examined expression of BDNF-associated miRNAs in rat brain and blood following either acute (x1) or chronic (x10) ECS. They focused primarily on those miRNAs that targeted BDNF. They found that level of one of the BDNF-related miRNAs miR-212 was significantly increased in rat dentate gyrus following both acute and chronic ECS. MiR-212 level was also increased in whole blood following chronic ECS and this was positively correlated with miR-212 levels in the dentate gyrus. These results suggest that alterations in miR-212 could be associated with BDNF modulation by ECS and that altered miR-212 expression in both blood and brain can be used as an indicator of ECS response.

O’Connor et al. (2013) investigated changes in hippocampal miRNAs induced by early-life stress (maternal separation) in rats and whether antidepressant treatments such as selective serotonin reuptake inhibitor (SSRI) fluoxetine, the rapid acting N-methyl-d-aspartate receptor antagonist ketamine, and electroconvulsive shock therapy (ECT) can reverse these changes. Microarray analysis revealed that early-life stress affected levels of 24 hippocampal miRNAs. When given to non-stressed animals, chronic fluoxetine treatment, repeated ECT, and acute ketamine treatment significantly altered the expression levels of 2, 10 and 14 miRNAs, respectively. One of these increases was common to all three antidepressants, namely miR-598-5p. Chronic fluoxetine treatment significantly decreased 4 miRNAs
following maternal separation with 3 of these representing a partial normalization of stress-induced changes. Repeated ECT altered 86 miRNAs (48 decreased, 38 increased); 16 of these were a normalization of stress-induced changes. Acute ketamine treatment altered 55 miRNAs (32 decreased, 23 increased); 11 of these changes were a reversal to stress-induced changes. ECT and ketamine treatment shared 43 common miRNA targets following maternal separation with seven being a reversal to stress-induced change. All three antidepressants shared one common miRNA target in maternal separated animals, i.e., miR-451, which showed reversal following stress-induced change. These results suggest that changes to hippocampal miRNA expression may represent an important component of stress-induced pathology and antidepressant action may reverse these changes.

In a PTSD mouse model, Schmidt et al. (2013) assessed miRNA profiles in prefrontal cortices dissected from either fluoxetine or control-treated wild-type C57BL/6N mice 74 days after their subjection to either a single traumatic electric foot-shock or mock-treatment. Screening for differences in the relative expression levels of all potential miRNA target sequences in PFC resulted in identification of 5 miRNA candidate molecules. Validation of these miRNAs revealed that the therapeutic action of fluoxetine in shocked mice was associated with a significant reduction in mmu-miR-1971 expression, suggesting that traumatic stress and fluoxetine interact to cause distinct alterations in the mouse PFC miRNA signature in the long-term. Although the functional significance of miR-1971 is not known at present, however, gene ontology analysis of predicted miR-1971 target genes suggested that this miRNA might be involved in basic metabolic processes like heterocyclic and organic substance metabolism.

**MiR-16: Regulator of Serotonin Transporter and its Involvement in Fluoxetine-induced Antidepressant Action:** It has been shown that SSRI antidepressant treatment reduces serotonin transporter (SERT) expression at protein level but does not affect SERT mRNA level (Benmansour et al., 2002). This suggests that SSRIs may interfere with SERT expression at the translation level. This led to the examination of miRNA-regulated expression of SERT. It was found that SERT is regulated by miR-16 (Baudry et al., 2010; Hansen and Obrietan, 2013). SERT is under inhibitory control of miR-16, which binds to its 3'UTR region and inhibits its expression. Interestingly, in mouse brain, Baudry et al. (2010) and Hansen and Obrietan (2013) demonstrated that miR-16 targets SERT in both raphe and locus coeruleus (LC). Treatment of mice with fluoxetine elevated the levels of miR-16 in serotonergic raphe nuclei and reduced SERT expression. Furthermore, the fluoxetine-mediated increase in

miR-16 level in raphe was accompanied by a decrease in pre/pri–miR-16 supporting the hypothesis that fluoxetine-induced up-regulation of miR-16 in raphe nuclei involved enhanced maturation from pre/pri-miR-16. How fluoxetine affects miR-16 maturation is not clear, however, it has recently been shown that Wnt signaling may play a crucial role in such maturation (Millan, 2011). Surprisingly, fluoxetine decreased the level of miR-16 in the noradrenergic LC, which was associated with the action of neurotrophic protein S100β released by raphe in response to SSRI treatment. These findings were further confirmed in the unpredictable chronic mild stress model (UCMS) such that mice exposed to a 6-week UCMS alleviated behavioral response to the same extent when infused with fluoxetine or miR-16 into raphe or by anti-miR-16 into the locus coeruleus (Launay et al., 2011). Fluoxetine-exposed serotonergic neurons also secreted BDNF, Wnt2 and 15-Deoxy-delta12,14-
prostaglandin J2. These molecules were unable to mimic the action of fluoxetine on their own but they acted synergistically to regulate miR-16 in hippocampus (Launay et al., 2011).

Putative miRNA binding sites in human SERT 3’UTR have also been predicted by bioinformatics analysis and validated by luciferase reporter assay experiments (Moya et al., 2012). Wild type, antisense, and various mutants of the 3’UTR were cloned downstream to the luciferase gene of reporter system. Then the luciferase-3’UTR constructs were co-transfected into rat raphe medullary raphe cells and human choriocarcinoma cells (expressing SERT) combined with miRNA mimics, inhibitors or negative miRNA as control. The data obtained from those reporter assay experiments indicate that not only miR-16 but miR-15a also plays a role in regulation of SERT expression in raphe nuclei. The effect of miR-15a was comparable to those of miR-16 reported in mice by Baudry et al. (2010). These findings represent a novel model for SERT expression regulation exerted by miR-15a/16 cluster adjacently located at human chromosome 13q14.3.

**Possible Therapeutic Application of miRNAs in Depression and other Psychiatric Illnesses**

As mentioned above, it appears that miRNAs are significantly altered in depressed individuals. Complementary studies come from animal model system, where individual miRNAs and their functional responses have been shown to be associated with many physiological functions in brain. Interestingly, circulating miRNAs are being studied in many psychiatric illnesses including depression as possible diagnostic tools. Although various psychoactive drugs, including fluoxetine and other antidepressants alter the expression of miRNAs, how these miRNAs are involved in their therapeutic response is not clear at the present time. But these studies provide at least reasonable proof that miRNAs can eventually be used as therapeutic targets. Several strategies have been shown to utilize miRNAs for therapeutic approaches, particularly in cancer biology. For example, miRNA oligonucleotides have been generated, which can directly compete with endogenous miRNAs. This strategy has been successfully in targeting specific miRNA (miRNA-21) and reducing its expression, which otherwise is overexpressed in several types of cancer (Si et al., 2007).

The other strategy to reduce miRNA expression is to employ locked-nucleic-acid antisense oligonucleotides or miR-masking, which requires a sequence with perfect complementarity to the target gene such that duplexing can occur with higher affinity than that between the target gene and its endogenous miRNA (Ebert et al., 2007). miRNAs can also be overexpressed. For this, adenovirus-associated virus containing specific miRNAs can be delivered to the target tissue (Kota et al., 2009).

In addition, miRNA mimics can be used to increase specific miRNA expression. These double-stranded RNA molecules mimic endogenous mature miRNAs (Landen et al., 2005). Thus, there are many strategies that can be used to inhibit or overexpress miRNA of interest. Several of these strategies are in pipeline for neurodegenerative diseases (Meng et al., 2013) but it will be interesting to see if these methods/strategies are useful in other complex disorders such as psychiatric illnesses.
Conclusion and Future Perspectives

By influencing a large number of target genes and regulating gene circuitry, miRNAs may have major implications in disease pathophysiology. As mentioned in Table 1, miRNAs affect many processes that are involved in MDD pathophysiology, including neural plasticity, neurogenesis, and stress response. In addition, direct studies in human postmortem brain as well as peripheral tissues further support these ideas.

MDD is a complex disorder and heterogeneity is inherently linked to this disease manifestation. Thus, before making any conclusive determinations, various clinical sub-phenotypes and confounding variables need to be carefully considered. For example, it will be interesting to examine whether changes in miRNAs are similar or dissimilar in melancholic vs. non-melancholic depressed patients. Also interesting will be to examine whether altered expression of miRNAs in depressed patients during adulthood are associated with childhood abuse. As indicated in animal model system, maternal separation leads to changes in several miRNAs. Whether these miRNAs play a role in development of MDD in later life is not known. It is noteworthy that recently, we have shown that enoxacin, a compound that stabilizes TRBP-Dicer complex prevented learned helpless behavior in rats (Smalheiser et al., 2014), suggesting that alterations in miRNAs may have phenotypic consequences.

There are many other avenues that need proper attention. For example, it will be worthwhile examining how miRNAs that are involved in MDD are dysregulated and if SNPs or CNVs play any role in such regulation. Also, an integrated view of miRNA network(s) and the pathways that are affected by these miRNAs need to be evaluated. This is important since not only individual miRNAs but combination of miRNAs provide more powerful regulatory mechanism of gene regulation. A set of miRNAs that are significantly affected in MDD and the corresponding set of mRNAs that are affected in the same samples will help resolve this issue.

As has been discussed earlier, presence of miRNAs biogenesis machinery in the synapse may regulate gene expression locally. Since MDD is associated with altered synaptic plasticity, it will be interesting to examine whether miRNAs are synthesized at the synapse in an activity-dependent manner and whether these miRNAs regulate synaptic proteins involved in MDD pathogenesis.

A variety of enzymes are responsible for processing miRNAs. These include drosha, dicer and cofactors DGCR8, TRBP, and PACT. Several of these proteins have been shown to be modified post-transnationally in a dynamic manner. For example, altering the relative expression of eIF2c may change the efficiency of translational arrest produced by a given miRNA. Recently, it has been shown that dicer is activated by proteolytic cleavage under conditions of elevated calcium levels (Smalheiser et al., 2008; Lugli, 2005) and eIF2C undergoes reversible phosphorylation within cells, which is required for its translocation to processing bodies (Zeng et al., 2008). The phosphorylation of eIF2C appears to be due to activation of ERK1/2 (Zeng et al., 2008). Since we have shown abnormalities in calcium-sensing proteins and ERK1/2 signaling in the brain of MDD subjects (Dwivedi 2001, 2011, 2006, 2009), it will be worthwhile examining whether dicer cleavage patterns or eIF2C phosphorylation are altered in the MDD subjects.
Table 1. miRNAs, their known targets, and functional relevance

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target</th>
<th>Known Effect/ Clinical Relevance</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>miR-124</td>
<td>Sox 9</td>
<td>Neuronal maturation, neurogenesis</td>
<td>Lagos-Quintana et al. 2002; Silber et al. 2008; Cheng et al. in 2009; Chen et al., 2009</td>
</tr>
<tr>
<td>miR-137</td>
<td>Ezh2</td>
<td>Neurogenesis, proliferation</td>
<td>Szulwach et al. 2010</td>
</tr>
<tr>
<td>miR-26a</td>
<td>Microtubule-associated protein 2</td>
<td>Neuronal differentiation, Neurogenesis</td>
<td>Kye et al. 2007</td>
</tr>
<tr>
<td>miR-134</td>
<td>Limk1</td>
<td>Regulates dendritic spine development</td>
<td>Schratt et al. 2006</td>
</tr>
<tr>
<td>miR-138</td>
<td>APT1</td>
<td>Regulates hippocampal dendritic spine development</td>
<td>Siegal et al. 2009</td>
</tr>
<tr>
<td>miR-132</td>
<td>P250GAP, MeCP2, CREB, SERT1, NR2A, NR2B, GLUR1</td>
<td>Induces neurite outgrowth, regulates dendritic morphology of cortical and hippocampal neurons, neuronal maturation, synapse Formation</td>
<td>Vo et al. 2005; Magill et al. 2010; Fukada et al. 2005; Jugloff et al. 2005; Klien et al. 2007; Mellios et al. 2011</td>
</tr>
<tr>
<td>miR-16</td>
<td>SERT</td>
<td>Neuronal maturation</td>
<td>Baudry et al. 2010; Hansen and Obrietan, 2013</td>
</tr>
<tr>
<td>miR-96, miR-141, miR-182, miR-183, miR-198, miR-200a, miR-200b, miR-200b*, miR-200c, miR-429</td>
<td>Creb1</td>
<td>Downregulated in Non-Learned Helplessness rats</td>
<td>Smalheiser et al. 2011</td>
</tr>
<tr>
<td>miR-142-5p, miR-33a, miR-137, miR-489, miR-148b, miR-101, miR-324-5p, miR-301a, miR-146a, miR-335, miR-494, miR-20b, miR-376a, miR-190, miR-155, miR-660, miR-552, miR-453, miR-130a, miR-27a, miR-497, miR-10a, miR-20a, miR-142-3p</td>
<td>VEGFA, BCL2, DNMT3B, MYCN, Various transcription factors and signaling proteins</td>
<td>Significantly downregulated in postmortem brain of depressed human patients who committed suicide, possibly due to decreased transcription</td>
<td>Smalheiser et al. 2013, 2014</td>
</tr>
<tr>
<td>miR-124, miR-139-5p, miR-195, miR-198, miR-320c, miR-33b, miR-34a, miR-34c-5p, miR-497, miR-873</td>
<td>SAT1, SMOX</td>
<td>Regulate polyamine gene expression in frontal cortex of human suicide patients</td>
<td>Lopez et al. 2013</td>
</tr>
<tr>
<td>miR-107, miR-133a, miR-148a, miR-200c, miR-381, miR-425-3p, miR-494, miR-517b, miR-579, miR-589, miR-636, miR-652, miR-941, and miR-1243</td>
<td></td>
<td>Altered expression in peripheral blood mononuclear cells of severe MDD patients</td>
<td>Belzeaux et al. 2012</td>
</tr>
<tr>
<td>let-7d, let-7e, miR-26a, miR-26b, miR-34c-5p, miR-103, miR-128, miR-132, miR-183, miR-192, miR-335, miR-494 and miR-22</td>
<td></td>
<td>Altered expression after 12 weeks of escitalopram treatment in MDD subjects, neural plasticity, stress response</td>
<td>Bocchio-Chiavetto et al. 2013</td>
</tr>
</tbody>
</table>

The presence of miRNAs in peripheral tissues, particularly, in blood cells provide promising approach to use miRNAs as potential biomarkers for both diagnosis and treatment
response. However, there are several issues that need consideration for the use of circulating miRNAs as biomarkers. For example, the source of miRNAs in blood cells is not clear at the present time. In this regard, profiling exosomal miRNAs derived from brain may prove useful exosomal miRNAs can be useful in detecting miRNAs. Finally, there is a possibility that changes in circulating miRNAs may not be directly related to the changes in brain. While this complicates the study of circulating miRNAs, it still holds promise, since it is likely that miRNA changes may reflect systemic alterations that accompany the disease process.

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References


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