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Evidence demonstrating role of microRNAs in the etiopathology of major depression

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Abstract

Major depression is a debilitating disease. Despite a tremendous amount of research, the molecular mechanisms associated with the etiopathology of major depression are not clearly understood. Several lines of evidence indicate that depression is associated with altered neuronal and structural plasticity and neurogenesis. MicroRNAs are a newly discovered prominent class of gene expression regulators that have critical roles in neural development, are needed for survival and optimal health of postmitotic neurons, and regulate synaptic functions, particularly by regulating protein synthesis in dendritic spines. In addition, microRNAs (miRNAs) regulate both embryonic and adult neurogenesis. Given that miRNAs are involved in neural plasticity and neurogenesis, the concept that miRNAs may play an important role in psychiatric illnesses, including major depression, is rapidly advancing. Emerging evidence demonstrates that the expression of miRNAs is altered during stress, in the brain of behaviorally depressed animals, and in human postmortem brain of depressed subjects. In this review article, the possibility that dysregulation of miRNAs and/or altered miRNA response may contribute to the etiology and pathophysiology of depressive disorder is discussed.

Keywords

microRNA; depression; neurogenesis; plasticity; stress; animal model of depression; human postmortem brain

1. Introduction

Depression is a major public health concern. The lifetime prevalence rate of depression is approximately 5% to 19% (Kessler et al., 1994); of those, 50% to 85% experience multiple episodes. Depression is a major symptom of suicidal behavior (Mann 1998; Soloff et al., 2000). Despite many years of research, the molecular and cellular mechanisms associated with depression are not clear.

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Conflict of interest statement

The author declares that there are no conflicts of interest.

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Overwhelming evidence points to altered synaptic and structural plasticity and neurogenesis in major depression. In fact, major depression may result from an inability of the brain to make appropriate adaptive responses to environmental stimuli because of impaired synaptic plasticity (Manji et al., 1998; Duman et al., 2000; Duman, 2002; Fossati et al., 2004). Support for this comes from a variety of studies in depressed subjects, demonstrating compromised structural and functional plasticity (Ongur et al., 1998; Roskolija et al., 2000; Rajkowska, 2000; Cotter et al., 2001; Miguel-Hidalgo and Rajkowska, 2002); changes in synaptic circuitry (Aganova and Uranova, 1992); decreased dorsolateral prefrontal cortical activity (Dolan et al., 1993; Drevets et al., 1998); impaired synaptic connectivity between the frontal lobe and other brain regions (Andreassen, 1997; Honer, 1999); changes in the number and shape of dendritic spines, the primary location of synapse formation (Toni et al., 1999; Hajszar et al., 2005); altered dendritic morphological features of neurons in the hippocampus; decreased length and number of apical dendrites (McEwen, 2000); neuronal atrophy and decreased volume of the hippocampus (Sheline, 2000; Sala et al., 2004; Frodl et al., 2006); decreased number of neurons and glia in cortical areas (Rajkowska et al., 2007); and spatial cognition deficits (Sackeim, 2001). In addition, major depression is associated with a negative impact on learning and memory (Horan et al., 1997; Bearden et al., 2006); and stress, a major factor in depression, hinders performances on hippocampal-dependent memory tasks and impairs induction of hippocampal long-term potentiation (Howland and Wang, 2008). These studies clearly demonstrate impaired structural and functional plasticity in major depression; however, the precise molecular and cellular nature of events that lead to such altered plasticity, which contributes to depressive behavior, remains unclear.

2. Altered intracellular signaling and gene expression in major depression

Adaptive responses in intracellular molecules and gene expression, together with the modulation of functional responses mediated by the phosphorylation of critical proteins participate in a major way in neural plasticity. In fact, it has repeatedly been demonstrated that major depression is associated with alterations in the expression of genes that are crucial in the maintenance of neural and structural plasticity. For example, several studies have reported decreased expression of brain-derived neurotrophic factor (BDNF) in depressed subjects and in brain of stressed animals (reviewed in Dwivedi, 2009). We and other investigators have also reported that growth factors, such as NGF, NT-3, and NT-4, and their cognate receptor tyrosine kinases (Trk) receptors are less expressed and less activated in the postmortem brain or peripheral tissues of depressed subjects (Dwivedi et al., 2005a; Otsuki et al., 2008; Dunham et al., 2009; Dwivedi et al., 2009a). These findings suggest that signaling pathways that are mediated through neurotrophins/Trks may play an important role in the pathophysiology of depression. Neurotrophic factors and the expression of molecules in signaling pathways, which are linked with Trks and participate in neural plasticity, such as extracellular signal-regulated kinases (ERKs) and phosphoinositide 3-kinase, are also altered in depressed subjects, in animal models of depression, and during stress. Most notable is the finding of hypoactivation of these pathways via altered expression of intermediary molecules, such as ERK1/2, MAP Kinase kinase1, ERK5, Rap-1, B-Raf, and Epac (Dwivedi et al., 2001; 2006a; 2006b; 2007; 2009b; Gourley et al., 2008; Qi et al., 2008; Todorovic, 2009; Yuan et al., 2010). In addition, the expression of protein kinases A and protein kinase C that are integral parts of adenylyl cyclase-cyclic adenosine monophosphate and phosphoinositide signaling systems, respectively, are down regulated in depressed suicide subjects (Pandey et al., 1997; Dwivedi and Pandey, 2008). These protein kinases are critical elements of stimulus-response coupling (Hyman and Nestler, 1993). One such critical effect of the stimulation of these kinases is the subsequent phosphorylation of transcriptional factors, including cyclic adenosine monophosphate response element binding protein (CREB). The CREB phosphorylation is linked to both norepinephrine- and serotonin (5-HT) linked cascades and represents a common target of action of noradrenergic

and serotonergic neurotransmission, implicated in depression. Phosphorylated CREB binds to cyclic adenosine monophosphate response element (CRE) in the promoter region of genes, and thereby regulates gene expression (Yamamoto et al., 1988). This represents an integrative set of mechanisms by which antidepressants, acting via either norepinephrine or serotonin, can target a common set of genes and their respective protein products (Gass and Riva, 2007). CREB is involved in learning and memory, and there is general consensus that impaired CREB-induced adaptive gene expression contributes to the pathogenesis of major depression (Duman et al., 2002). This is evident from studies showing decreased phosphorylation and expression of CREB in brain of depression subjects (Chen et al., 2001; Dwivedi et al., 2003a). Another molecule that is gaining attention in depressive pathophysiology is vascular endothelial growth factor A (VEGFA). VEGF is an angiogenic cytokine, which induces vasopermeability in many types of tissues (Ferrara et al., 2003), including the blood-brain barrier (Rigau et al., 2007) and facilitates neurogenesis and proliferation of neurons in the adult hippocampus (Palmer et al., 2000). By inhibiting apoptosis and inducing growth of the associated vascular-neuronal networks, VEGF can act as a neuroprotective factor in the adult brain, (Brockington et al., 2004; Raab and Plate, 2007). VEGF also influences synaptic plasticity in hippocampus-dependent processes, such as learning and memory (Cao et al., 2004), and modulates synaptic transmission (McCloskey et al., 2005). Because of evidence of comorbidity between major depression and cardiovascular disease, many recent studies have examined VEGF in depression and found that VEGF mRNA level is altered in peripheral leukocytes of depressed patients, which is reversed by antidepressant treatment (Tanis et al., 2007; Malberg and Monteggia, 2008). This reversal parallels the clinical improvement (Iga et al., 2007). From these studies, it appears that the expression of genes that are an integral part of intracellular signaling cascades and involved in neural plasticity may play a major role in the pathophysiology of major depression.

3. MicroRNAs

3.1. General aspect

In the past few years, it has become clear that gene expression in the brain is regulated at many levels. In addition to the traditional transcriptional mechanisms (e.g., activation of genes by transcription factors and alternative splicing), gene expression is regulated by a variety of noncoding RNA transcripts that generate microRNAs (miRNAs), antisense RNAs, and other small RNAs that are linked to several posttranscriptional, transcriptional, and epigenetic mechanisms. Of these, the best investigated are the miRNAs, which were first identified as regulators of developmental timing and cell fate and later were found to comprise a population of hundreds of distinct genes that are widely expressed in nearly all multicellular organisms and in many tissues, including adult mammalian brain (Kosik, 2006; Bushati and Cohen, 2007). In recent years, the involvement of miRNAs in neurodevelopmental and brain disorders has gained momentum as many studies have focused on examining the role of miRNAs in Fragile X syndrome, Rett syndrome, DiGeorge syndrome, Down syndrome, autism, schizophrenia, Alzheimer disease, neurodegenerative disorders, and Huntington disease. However, studies of miRNAs in affective illnesses and particularly, in major depression are still in infancy. Nevertheless, recent studies, directly or indirectly, indicate that miRNAs have a potential to participate in a major way to contribute to the pathophysiology of major depression, particularly at the level of synaptic plasticity, neurogenesis, and by regulating expression of genes that are critical components of signaling pathways, implicated in major depression.

3.2. Biogenesis of microRNA

For detailed review on this aspect, please consult other excellent reviews (Ross et al., 2007; Kwak et al., 2010; Qurashi and Jin, 2010; Varol et al., 2011). A diagram showing miRNA biogenesis is depicted in Figure 1. In general, miRNAs are encoded within primary miRNA (pri-miRNA) gene transcripts that may be intergenic (away from known protein-coding genes) or may be located within introns of protein-coding host genes. miRNA genes are transcribed to long primary miRNA by RNA polymerase II or III. The pri-miRNAs are then processed further within the nucleus to form one or a series of small hairpin miRNA precursors, or precursor miRNAs (pre-miRNAs), that are generally 70 to 110 nt long and fold into a stem-loop structure. The protein that converts pri-miRNA into pre-miRNA is an RNase III enzyme Drosha. Generally, Drosha requires DiGeorge syndrome critical region 8 (DGCR8) protein as a cofactor for activation. Together with DiGeorge, Drosha forms a large complex known as the “microprocessor complex.” Drosha removes the flanking segments and ~11 bp stem region of the pri-miRNA. A small group of intronic miRNAs, also known as mitrons, is generated from hairpin introns. In the biogenesis of mitrons, the process of pri-miRNA cleavage mediated by Drosha/DGCR8 is bypassed. Instead, pre-miRNA is provided from introns by the nuclear splicing machinery. The pre-miRNAs are then transported out of the nucleus via the exportin transfer system, which consists of Exportin 5 and RanGTP. Pre-miRNA is released to cytoplasm upon hydrolysis of GTP to GDP. The pre-miRNAs are further processed in the cytoplasm by RNase III enzyme Dicer, which converts pre-miRNA into double-stranded mature small RNA (miRNA/miRNA* duplexes) of approximately 22 nt long. Dicer requires cofactors such as TAR RNA-binding protein (TRBP) or PKR-activating protein (PACT). 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.

3.3. Mechanisms of microRNA-mediated translational processes

The RISC complex binds to specific “short-seed” sequences located predominantly within the 3'-UTR region of mRNAs and can interfere with translation of the mRNA and/or may reduce mRNA levels. Different mechanisms have been proposed of the translational repression mediated by miRNAs: i) mRNA degradation: This occurs when the miRNA-RISC binds to the 3'UTR of the target mRNA, which are near exact complementary to each other. In this case, Ago, which has splicer function, induces mRNA degradation. ii) miRNA-RISC-mediated repressed formation of translational initiation complexes: In this case, Ago competes with eIF4E for binding to 7-methylguanosine (7mG) cap and represses formation of the translational initiation complex when it is recruited to the 3'UTR of a target mRNA by miRNA. This occurs because Ago protein within the miRNA-RISC complex shows structural homology with eIF4E. eIF6, which has been shown to be part of the Ago-RISC complex, can also repress translation. eIF6 can block the association of 40S and 60S ribosomes and inhibits the assembly of the 80S competent ribosome. iii) mRNA Translational repression by “P body”: In this case, RISC recruits target mRNA to processing bodies, in which mRNA is degraded or stored in a translationally inactive state. P bodies contain 5' to 3' and 3' to 5' exonucleases, decapase, and deadenylase, which are responsible for mRNA degradation.

Besides regulating translational process, 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). However, this could also lead to upregulation of genes if the transcription factors are inhibitory in nature. miRNAs tend to act in coregulated groups and to affect groups of targets as part of larger gene expression networks that contain feedback and feed-forward regulatory loops (Tsang et

al., 2007). Zhou et al., (2007) studied coordinated regulation of genes by miRNAs and transcription factors and found that many gene sets are reciprocally regulated by strongly interacting pairs of transcription factor-miRNA, implicating an efficient mechanism to suppress functionally related proteins. In addition, a particular type of feed forward loop (with two opposing modes where the transcription factor [TF] activates its target genes or the miRNA simultaneously suppresses this TF and the TF-miRNA joint target genes) is more prevalent among strongly interacting transcription factor-miRNA pairs, which may be attributed to a process that prevents waste of cellular resources or a mechanism to accelerate mRNA degradation.

In addition to the direct sequence-specific interaction of RISC with mRNAs, other proteins that bind nearby sites within the 3'-UTR (eg, FMRP homologues, HuB family members, and other ARE-binding proteins) may control the magnitude and even the direction of miRNA effects. Indeed, in certain situations (e.g., depending on the phase of the cell cycle in dividing cells, which possibly reflects reversible phosphorylation or methylation of FMRP homologues), miRNAs may actually enhance, rather than inhibit, translation (Vasudevan et al., 2007). In fact, several reports suggest miRNAs can target promoters and induce increases in levels of mRNA and protein (Li et al., 2006; Place et al., 2008).

3.4. Expression of microRNAs in CNS

Human miRNAs have a high copy number per cell, ranging from 1000 to 30,000, suggesting the critical role of miRNAs in regulating expression of a large number of genes. A single miRNA can regulate several mRNAs. Conversely, several miRNAs can regulate a single mRNA. Thus far, approximately 1000 miRNAs have been identified in the human genome (Wang and Wu, 2010; MiRBase, 2010) and approximately 30% of human genes are regulated by these miRNAs (Croce, 2009; Yendamuri and Calin, 2009). The expression of miRNAs is tissue specific and, in some cases, even cell specific (Landgraf et al., 2007; Kapsimali et al., 2007; Choi et al., 2008). In addition, some of the miRNAs are expressed specifically at the developmental stages (Ambros, 2004; Bartel, 2004). Approximately 20% to 40% of miRNAs in the brain are developmentally regulated, such that their expression increases in parallel to the brain development (Miska et al., 2004; Sempere et al., 2004). There are studies, which suggest that miRNAs, such as miR-124 and miR-128, are primarily expressed in neurons, whereas miR-23, miR-26, and miR-29 are expressed in high amounts in astrocytes (Smirnova et al., 2005). Given their differential nature of expression, the role of miRNAs in numerous biological phenomena, including neuronal development, stem cell proliferation, the cell cycle, and apoptosis, has been studied (Presutti et al., 2006; Fiore et al., 2008; Vreugdenhil and Berezikov, 2009). During adulthood, miRNAs also function in local regulation of mRNA translation and participate in neurogenesis, synaptic development, axon guidance, and neuronal plasticity (Presutti et al., 2006; Fiore et al., 2008; Liu and Zhao, 2009; Vreugdenhil and Berezikov, 2009). In the subsequent sections, the role of miRNAs in neurogenesis and synaptic plasticity and their direct role in depressive illness are discussed.

4. Role of microRNAs in neurogenesis and synaptic plasticity

4.1. microRNAs and neurogenesis

Numerous studies suggest that neurogenesis is involved in learning. For example, neurogenesis in the subventricular zone is important for olfactory learning (Alonso et al., 2006), whereas hippocampal neurogenesis is involved in memory and spatial learning (Dupret et al., 2008). Enriched environment, exercise, and antidepressant treatment all increase hippocampal neurogenesis (reviewed in Lazarov et al., 2010). In contrast, stress and major depression cause decreased hippocampal neurogenesis (reviewed in DeCarolis and

Eisch, 2010; Lucassen et al., 2010). The molecular factors regulating adult neurogenesis are currently under investigation, and how miRNAs-mediated regulation of neurogenesis can contribute to major depression remains to be seen. Nonetheless, several recent studies demonstrate that miRNAs not only play a major role in embryonic but also in adult neurogenesis. For example, Choi et al (2008) demonstrated that olfactory tissues express more than 100 distinct miRNAs, the most abundant being the miR-124a and let-7 variants and the family of miR-200. To determine whether miRNAs are required during olfactory neuronal development, Choi et al. (2008) analyzed embryonic tissues in which Dicer function was specifically ablated in olfactory progenitor cells. They showed that loss of miRNA function from olfactory progenitor cells produced no detectable alterations in patterning. In contrast, they noted that terminal differentiation of the olfactory progenitor pool into mature olfactory neurons did not occur and that the olfactory precursor cell population is not maintained. On the other hand, Cheng et al. (2009) showed that miR-124 is an important regulator of the temporal progression of adult neurogenesis. They found that knockdown of endogenous miR-124 maintained purified subventricular zone stem cells as dividing precursors, whereas ectopic expression led to precocious and increased neuron formation in mice. They identified the SRY-box transcription factor Sox9 as being a physiological target of miR-124 at the transition from the transit-amplifying cell to the neuroblast stage. The overexpression of Sox9 abolished neuronal differentiation, whereas Sox9 knockdown led to increased neuron formation. Thus, miR-124-mediated repression of Sox9 is important for progression along the subventricular zone stem cell lineage to neurons. More recently, Szulwach et al. (2010) showed that MeCP2, a DNA methyl-CpG-binding protein, can epigenetically regulate miR-137, which is overregulated by Sox2, a core transcription factor in stem cells. They found that miR-137 modulated the proliferation and differentiation of adult neural stem cells. The 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 posttranscriptionally 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 trimethyl lysine 27. Coexpression of Ezh2 rescued phenotypes associated with miR-137 overexpression, thus demonstrating a cross talk between miRNA and epigenetic regulation in the modulation of adult neurogenesis.

4.2. microRNA and Synaptic Plasticity

In the mammalian forebrain, local control of protein synthesis near synapses is critical for normal learning and dendrite spine morphogenesis (Vanderklish and Edelman, 2005). Increasing evidence points toward a major role of miRNAs in regulating the synaptic plasticity. This is achieved primarily by affecting the translation and/or stability of mRNA (Kosik, 2006; Gao, 2008; Christensen and Shratt, 2009). The evidence of the role of microRNAs in synaptic plasticity is derived from studies showing that FMRP, which regulates protein synthesis in dendritic spines, interacts with RISC components Ago1 and Dicer (Jin et al., 2004). Loss of FMRP produces a wide spectrum of behavioral, learning, dendritic spine, and long-term potentiation (LTP) deficits. The FMRP regulates protein synthesis by binding to specific sites within the 3'-UTR of certain mRNAs and probably does so in concert with miRNAs and the RISC complex (Qurashi et al., 2007). Both FMRP and RISC complex components are localized in the somatodendritic compartment (Caudy et al., 2002; Ishizuka et al. 2002), and FMRP associates strongly with several components of the miRNA machinery, including mature miRNAs, pre-miRs, dicer, and eIF2c. In *Drosophila*, Ashraf et al. (2006) demonstrated that synaptic expression of calcium/calmodulin-dependent protein kinase II α (CaMKII α) is negatively regulated by the RISC complex. In addition, one of the RISC proteins, Armitage, is necessary for long-term potentiation and synaptic protein synthesis and is cleaved during the learning process

(Ashraf et al., 2006). They showed that 3'-UTR of CaMKII α has binding sites for miR-280 and miR-289. More recently, Banerjee et al. (2009) reported that Mov 10, an RNA helicase that associates with Argonaute protein, is present at synapses and is rapidly degraded by the proteasome in an NMDA receptor-mediated activity-dependent manner. When Mov 10 was suppressed, a set of mRNAs, including CaMKII α , selectively entered the polysome compartment, suggesting a local translational regulatory mechanism during synaptic plasticity.

Schratt et al. (2006), for the first time showed that individual miRNA can regulate local protein synthesis in mammalian dendrites. They found that a brain-specific miRNA, miR-134, is localized to the synaptodendritic compartment of rat hippocampal neurons and can negatively regulate the size of dendritic spines (postsynaptic sites of excitatory synaptic transmission). They further demonstrated that this effect is mediated by miR-134 inhibition of the translation of an mRNA encoding a protein kinase, Limdomain-containing protein kinase 1 (Limk1), a protein that regulates dendritic spine growth. Exposure of neurons to BDNF relieves miR-134 inhibition of Limk1 translation, causing enhanced growth of dendritic spines. This derepression involves TrkB and mammalian target of rapamycin (Schratt et al., 2004).

In a similar manner, Siegel et al. (2009) found that miR-138 is highly enriched in the brain, is localized within dendrites, and negatively regulates the size of dendritic spines in rat hippocampal neurons. The miR-138 controls the expression of acyl protein thioesterase 1, an enzyme regulating the palmitoylation status of proteins that are known to function at the synapse, including G proteins (G α 13). RNA interference-mediated knockdown of acyl protein thioesterase 1 and expression of membrane-localized G α 13 both suppress spine enlargement caused by inhibition of miR-138, suggesting that acyl protein thioesterase 1-regulated depalmitoylation of G α 13 might be an important downstream event of miR-138 function.

The Smalheiser group recently showed that a subset of miRNAs is specifically enriched in synaptic fractions isolated from adult mouse forebrain (Lugli et al., 2008). Both dicer and pre-miRNAs are expressed in synaptic fractions and are strongly associated with postsynaptic densities, suggesting that at least a portion of the mature miRNAs is locally processed near synapses. Indeed, dicer is released from postsynaptic densities and its RNase III activity is markedly enhanced, following stimuli such as NMDA that cause an increase in local calcium and activation of calpain (Lugli et al., 2005, 2008). Calpain cleaves dicer and may act, in part, by removing an N-terminal inhibitory domain.

In a further attempt to examine the role of miRNAs in local protein synthesis, Lugli et al. (2008) characterized the expression of miRNAs and selected miRNA precursors within several synaptic fractions of adult mouse forebrain, including synaptoneurosome, synaptosomes, and isolated postsynaptic densities. They found that most brain miRNAs (especially miRNAs known to be expressed in pyramidal neurons) are detectably expressed in synaptic fractions and that a subset of miRNAs is significantly enriched in synaptic fractions relative to total forebrain homogenate. miRNA precursors were also detectable in synaptic fractions at levels that are comparable to whole tissue. Although mature miRNAs were predominantly associated with soluble components of the synaptic fractions, miRNA precursors were predominantly associated with postsynaptic densities. There was a significant correlation between the relative synaptic enrichment of the precursor and the relative synaptic enrichment of the corresponding mature miRNA. These findings suggest that miRNAs are formed, at least in part, by processing of miRNA precursors locally within dendritic spines. Dicer is expressed in postsynaptic densities but is enzymatically inactive

until conditions that activate calpain cause its liberation. They propose that synaptic stimulation may lead to local processing of miRNA precursors in proximity to the synapse.

CREB, a key protein regulating long-term changes in gene expression, is among a set of major hubs for miRNA regulation, insofar as their 3'-UTR regions contain "short-seed" recognition sites for more than 40 miRNAs. Conversely, many miRNA genes have binding sites in their promoter regions for Creb1 (Vo et al., 2005; Wu and Xie, 2006). For example, miR-132 is activity dependent and induced by BDNF acting via CREB. It has been shown that neuronal activity regulates spine formation, in part, by increasing miR-132 transcription (Impey et al., 2010). Overexpression of miR-132 enhances neurite outgrowth and dendritic morphogenesis (Vo et al., 2005; Wayman et al., 2008). In addition to miR-132, miR-124 has been found to be ubiquitously and constitutively expressed in most neuronal cell types of the mammalian brain, and play a primary role specifying and maintaining neuronal cell identity. Interestingly, neuromodulators act through miRNAs during various forms of plasticity and this may occur locally at synapses. For example, Rajasethupathy et al. (2009) found that miR-124 is rapidly and robustly regulated by 5-HT. They reported that 5HT selectively affects mature miR-124 levels, without affecting its precursor, suggesting that the miR-124 level may be regulated during Dicer processing or RISC incorporation and stabilization by Argonaute. One of the major targets of miR-124 is CREB, and miR-124 responds to 5-HT by de-repressing CREB and enhances 5-HT-dependent long-term facilitation (Rajasethupathy et al., 2009). More recently, *SIRT1*, a gene relevant in oxidative stress and circadian rhythms (Nakahata et al., 2008; Finkel et al., 2009), was shown to modulate synaptic plasticity and memory formation via posttranscriptional regulation of CREB expression by brain-specific miRNA miR-134 (Gao et al., 2010). The SIRT1 limited the expression of miR-134 via a repressor complex containing the transcription factor YY1. Unchecked miR-134 expression after SIRT1 deficiency resulted in the reduced expression of CREB and BDNF, thereby impairing synaptic plasticity. The knockdown of miR-134 rescued the LTP and memory impairment caused by SIRT deficiency.

To address the impact of loss of miRNAs in fully differentiated adult neurons, Konopka et al. (2010) used an inducible variant of the Cre recombinase (tamoxifen-inducible CreERT2) under control of *CaMKII α* gene regulatory elements. After induction of *Dicer1* gene deletion in adult mouse forebrain, they observed a progressive loss of a whole set of brain-specific miRNAs, along with enhancement of cognition in adult *Dicer1*-mutant mice. In these animals, they noted increased translation of proteins, such as BDNF and MMP-9 that have been involved in synaptic morphology.

4.3. BDNF and microRNAs

One of the signaling molecules that plays a crucial role in synaptic and neural plasticity, is BDNF. Several studies have demonstrated that BDNF regulates structural, synaptic, and morphological plasticity to modulate the strength or number of synaptic connections and neurotransmission (reviewed in McAllister et al., 1999; Thoenen, 2000). In addition, BDNF participates in cellular proliferation, migration, phenotypic differentiation, and/or maintenance in the developing central nervous system (McAllister, 2001). In addition, its presence is required in the adult central nervous system for maintenance of neuronal functions and neurogenesis (Cooper et al., 1996; Sofroniew et al., 1990). Thus, a pathological alteration of the BDNF may not only lead to defects in neural maintenance and regeneration and, therefore, structural abnormalities in the brain, but may also reduce neural plasticity and, therefore, impair the individual's ability to adapt to crisis situations. The role of BDNF in major depression gained broad attention because many pre-clinical and clinical studies provided direct evidence showing that modulation in the expression of BDNF could be involved in behavioral phenomena associated with major depression (Smith et al., 1995; Dwivedi et al., 2003b; Karege et al., 2005; Brunoni et al., 2008; Castrén and Rantamäki,

2008; Kozisek et al., 2008; Dwivedi, 2009; Fanous et al., 2010; Shi et al., 2010). Based on these studies, the neurotrophin hypothesis of major depression was proposed, suggesting that stress and depression are associated with decreased expression of BDNF and that antidepressants alleviate depressive behavior by increasing its level (Duman et al., 2000; Duman, 2002;). Recently, Kumamaru et al. (2008) and Numakawa et al. (2009) reported that glucocorticoids suppress BDNF-induced synaptic maturation and excitatory neurotransmitter glutamate release, whereas antidepressants reinforced BDNF-triggered glutamate release (Yagasaki et al., 2006). These findings suggest that the prevention of BDNF action by increased glucocorticoid is closely related to depressive disorders. Interestingly, BDNF regulates the expression of miR-132, which occurs via CREB. In fact, the overexpression of miR-132 causes enhanced neurite outgrowth and dendritic morphogenesis (Vo et al., 2005; Wayman et al., 2008). This was confirmed by Kawashima et al. (2010), who showed that BDNF selectively up regulated the expression of miR-132 in cultured neurons, whereas no change occurred in the expression of other miRNAs, such as miR-9, miR-124, miR-128a, miR-128b, miR-134, miR-138, and miR-16. In addition, the transfection of exogenous ds-miR-132 induced marked up regulation of glutamate receptors (NR2A, NR2B, and GluR1, suggesting that miR-132 has a positive effect on the increase in postsynaptic proteins levels. Interestingly, inhibition of the ERK pathway suppressed the BDNF-increased miR-132, suggesting that up regulation of miR-132 by BDNF occurs via the ERK1/2 pathway. On the other hand, pretreatment with glucocorticoid reduced BDNF-increased ERK1/2 activation, miR-132 expression, and postsynaptic protein levels. These results provide a mechanism by which BDNF and ERK1/2 regulate miR-132 expression and suggest that the exposure of neurons to an excess glucocorticoid causes decreased BDNF-dependent neuronal functioning by suppressing miR-132 expression. It is pertinent to mention that both BDNF and ERK1/2 have been implicated in stress and depression. Interestingly, BDNF itself is a target of several miRNAs. For example, miR-30a and miR-195 directly target the BDNF 3'-UTR, thus reducing its expression (Mellios et al., 2008). Whether such mechanisms occur at hyperactive hypothalamus-pituitary-adrenal (HPA) axis or in stress-induced pathological conditions, remains to be studied.

5. microRNAs and Depression

5.1. microRNAs and glucocorticoid receptors

Several clinical and epidemiological studies have identified stressors as important risk factors in depression (reviewed in Liu and Alloy, 2010). An overactive HPA axis is well established in stress and abnormal HPA axis in depression is one of the most consistent findings in biological psychiatry (reviewed in Wasserman et al., 2010). Most patients with depression have increased concentrations of cortisol in plasma and cerebrospinal fluid, an increased cortisol response to adrenocorticotrophic hormone, and a deficient feedback mechanism, as evidenced by an abnormal dexamethasone suppression test result and enlarged pituitary and adrenal glands. The release of corticotropin-releasing factor from the paraventricular nucleus of the hypothalamus causes the release of adrenocorticotrophic hormone from the pituitary, which stimulates the production of glucocorticoids (cortisol in humans and corticosterone in animals) from the adrenals. Corticosterone binds to two different types of receptors: glucocorticoid receptors (GRs) and mineralocorticoid receptors (MRs). A fine balance between these two receptors is important for homeostasis and mental stability. Any disturbance could lead to impaired behavioral adaptation and stress-related diseases (reviewed in de Kloet, 2009). Glucocorticoids regulate the HPA axis through a negative feedback mechanism while binding to soluble GRs in the pituitary and the hypothalamus and inhibiting the release of corticotropin-releasing factor and adrenocorticotrophic hormone. Several studies have reported that expression of GRs is down regulated in depressed individuals (reviewed in Pariante and Miller, 2001).

Under in vivo conditions, Uchida et al. (2008) characterized the vulnerability to repeated stress in F344 rats and found that expression of GR protein is much lower in the paraventricular nucleus (PVN) of these rats. Interestingly, they found that miR-18a expression is greater in the PVN of these rats. Functional analysis revealed that miR-18a inhibited translation of GR mRNA, suggesting that a decrease in GR may be, at least in part, because of the increased miR-18a expression.

In another study, Vreugdenhil et al. (2009) investigated the role of miRNAs in regulating GR protein expression. They tested a panel of miRNAs consisting of 124a, 328, 524, 22, and 18 and found that two miRNAs, (ie, miR-124a and miR-18a) were able to reduce GR protein levels. The miRNA reporter assays revealed binding of miR-124a to the 3'-UTR of GR and overexpression of miR-124a and miR-18a attenuated glucocorticoid-induced leucine zipper, a gene induced by stress-like levels of glucocorticoid. These studies indicate that GR expression is under control of specific miRNAs and indicate that miR-18a- and miR-124a-mediated down regulation of GR translation may be important factors in susceptibility to stress-related disorders. More recently, Turner et al. (2010) predicted several possible miRNA binding sites within the GR first exon. Examining these binding sites will further provide in-depth information on the regulation of GR via miRNAs.

5.2. Stress response and microRNAs

Restraint stress is one of the most commonly used animal models of stress and has been shown to modulate plasticity-related neuronal processes that might play a key role in stress-induced vulnerability to affective illnesses. Rinaldi et al. (2010) investigated the effects of restraint stress on miRNA expression in the brain of mice. They found that restrained stress induced a transient increase in the expression of selected miRNA in the frontal cortex, which was region specific, as no alterations of miRNA levels were observed in the hippocampus. More specifically, they found that expression of miR-9, miR-9*, miR-26b, miR-29b, miR-30b, miR-30c, miR-30e, miR-125a, miR-126-3p, miR-129-3p, miR-207, miR-212, miR-351, miR-423, miR-487b, miR-494, miR-690, miR-691, miR-709, miR-711, and Let-7 a-e were increased in the frontal cortex of mice after acute stress. When selected miRNAs (let-7a, miR-9, miR-26a/b, miR-30b/c, and miR-125a) were further examined in the frontal cortex and hippocampus after acute and repeated stress and 5 days after acute stress, a significant increase in the expression of let-7a, miR-9, and miR-26a/b was observed in the frontal cortex of mice with acute stress as compared with naive mice. The miR-9, let-7a, and miR-26a/b expression levels were not altered after repeated restraint or 5 days after acute stress. These results suggest that acute stress modulates miRNA expression quickly because its effects are not long lasting and lead to the possibility that changes of miRNA levels after restraint stress might allow frontal neurons to quickly respond to external stimuli by changing their synaptic efficacy through regulation of localized mRNA translation.

To test if stress-responsive brain regions display altered miRNA profiles following psychological stress, Meerson et al. (2009) examined miRNA expression in the central amygdala and the CA1 region of the hippocampus of rats subjected to acute or chronic immobilization stress. They found that the expression of several miRNAs was differentially altered during acute and chronic stress, with chronic stress causing much larger changes than acute stress. In the central amygdala, 10 miRNAs were upregulated or down-regulated under acute stress, compared with chronic stress, for which alterations in 28 miRNAs were observed. Similarly, the hippocampal CA1 region showed 16 and 22 modified miRNAs after acute and chronic stress, respectively. Some of the miRNAs that were altered during acute or chronic stress include miR-134, miR-183, miR-132, Let-7a-1, miR-9-1, and miR-124a-1. Interestingly, the expression profiles of stress-responsive miRNAs were different in the two analyzed brain regions, except for miR-Let-7a-1, which was the only miRNA affected by both acute and chronic stress in the central amygdala. In the hippocampal CA1 region,

miR-376b and miR-208 both increased under acute or chronic stress, whereas miR-9-1 decreased under both of these conditions. miR-134 and miR-183 are predicted to target many splicing factors, such as SC35, SRP46, and SFRS11. Further studies by these investigators revealed that knockdown of miR-183 expression increased SC35 protein levels in vitro, whereas overexpression of miR-183 reduced SC35 protein levels. SC35 promotes the alternative splicing of acetylcholinesterase from the synapse-associated isoform AChE-S to soluble AChE-R protein and the expression of SC35 is increased during stress. Thus, by regulating splicing factors and their targets, miR-183 and miR-134 may modify both alternative splicing and cholinergic neurotransmission in the stressed brain. In addition, one of the targets of miR-183 is profilin 2 mRNA, which regulates dendritic spine morphology in neurons. Interestingly, neurotransmitter homeostasis and behavior are severely affected in profilin 2 knockdown mice (Witke, 2004) and PFN2 expression is increased in lymphoblastoid cell lines of monozygotic twin pairs discordant for bipolar disorder (Matigian et al. 2007).

The role of miRNAs in early-life stress, which may act as a predisposing factor for affective and anxiety disorders, has also been studied. Uchida et al. (2010) found that maternal separation enhanced stress vulnerability to repeated restraint stress exposure in adulthood. At the molecular level, maternal separation increased the expression of Repressor element-1 silencing transcription factor (REST) 4 mRNA and protein. The REST is a transcription factor that binds to the RE-1-conserved consensus sequence, allowing the transcriptional repression of RE-1-containing target genes. Interestingly, they found that transient overexpression of REST4 in the medial prefrontal cortex of neonatal mice produced depression-like behaviors in adults after repeated exposure to restraint stress, suggesting that REST4 may play an important role in the development of stress vulnerability. In addition, they found that the expression of several genes that contain RE-1 sequences in their promoter regions was altered in the medial prefrontal cortex of these stressed rats. Specifically, the expression levels of AMPA glutamate receptor 2 subunit (*Glur2*), NMDA receptor 1 subunit (*Nr1*), *Crh*, *CaMKIIa*, cell adhesion molecule L1, adenylate cyclase 5, *5hr1a*, and voltage-gated potassium channel subunit Kv3.1 were up regulated; some of these genes have been shown to be involved in depression and anxiety. Because REST also regulates the expression of several brain-enriched miRNAs (Conaco et al., 2006; Otto et al., 2007) that are involved in brain development and plasticity (Vo et al., 2005; Kosik, 2006; Rajasethupathy et al., 2009), Uchida et al. (2010) examined whether maternal separation alters the expression of these miRNAs. They found that expression levels of REST-controlled pre-miRNAs 132, 124-1, 9-1, 9-3, 212, and 29a were significantly increased in the medial prefrontal cortex of these stressed rats. As is known that miRNAs miR-132 and miR-124 participate in synaptic plasticity (Vo et al., 2005; Rajasethupathy et al., 2009) and cause decreased dendritic length (Pascual et al., 2007), high synaptic density (Ovtscharoff and Braun, 2001), and altered basal neuronal activity (Stevenson et al., 2008). Thus, the findings that maternal separation increased REST4 and caused alterations in various regulated genes and miRNAs appear to be crucial in increasing the risk for the development of stress-related disorders, such as depression, in adulthood.

5.3. microRNAs and stress-induced behavioral depression

Because the ability to cope with stress is critical at the human level, parallel studies of the effects of uncontrollable stress have been performed in animals, with results of proactive interference with the acquisition of escape/avoidance responses (Seligman and Maier, 1967). This phenomenon is termed learned helplessness (LH) and has been used most extensively as an animal model of stress-induced behavioral depression. One of the limitations of the LH model is that the behavioral changes after a single shock persist only for a short period (24 hours). On the other hand, by definition, depression persists much longer after negative life

events. To overcome this issue, my group modified the LH model to include repeated shock sessions, which greatly prolonged the duration of LH, retaining all the behavioral features that have been induced in the single-shock LH model. This represents a significant advance over the single-shock LH paradigm because the responsive mechanisms and neurochemical changes may be different in acute and recurrent depression. In previous studies, we found subtle neurochemical differences in the acute vs. repeated LH model (Dwivedi et al., 2004, 2005b, 2005c); and treatment with desipramine (a norepinephrine uptake blocker) and fluoxetine (a 5-HT uptake blocker) for 14 days reversed LH behavior and the LH-induced decrease in hippocampal neurogenesis (Chen et al. 2006).

In this LH animal model, we determined the profiling of miRNA expression in the frontal cortex of LH, NLH, and sham rats to learn whether miRNA expression is altered specifically in the LH vs. NLH condition and whether this is part of a larger coordinated response to repeated shocks that is related to the depression-like phenotype (Smalheiser et al., 2011). To create the repeated LH model, inescapable shock consisted of 100 random shocks were delivered for 5 seconds at 1.0 mA, with a mean interval of 60 seconds on days 1 and 7. Another group of rats was placed in Plexiglas tubes but is not subjected to shocks (tested controls). Escape latencies were tested on days 1 and 8. Rats were divided into two groups based on mean latency: 1) rats in which the mean latency was 20 seconds or longer (LH) and 2) rats in which the mean latency was shorter than 20 seconds (non-LH [NLH]). With this protocol, we have found that approximately 50% of subjects become LH rats. The rats that were confined to Plexiglas tubes but were not shocked were also tested and termed as tested controls. We have repeatedly demonstrated that the rats that are tested on day 8 and show LH behavior after a second inescapable shock remain LH on day 14 (Dwivedi et al., 2004, 2005b; 2005c). We found that NLH rats showed a robust adaptive miRNA response to inescapable shock whereas LH rats showed a markedly blunted response. One set of miRNAs showed particularly large, significant, and consistent alterations in NLH rats, consisting of 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. All were down regulated in NLH rats relative to tested controls, and all showed a blunted response in LH rats. All were encoded at a few shared polycistronic loci, suggesting that their down regulation was coordinately controlled at the level of transcription. Most of these miRNAs have previously been shown to be enriched in synaptic fractions (Lugli et al., 2008). Moreover, almost all of these miRNAs share 5'-seed motifs with other members of the same set, suggesting that they will hit similar or overlapping sets of target mRNAs. Several of the miRNAs in this set have experimentally validated targets; notably, miR-200a,b,c and 429 are known to target the transcription factor Zeb1 and Zeb2 and, in turn, Zeb1-binding sites are present upstream of miR-96, 141, 182, 183, and 200c, stimulating their expression and forming a complex feedback loop (Bracken et al., 2008). Finally, half of this set are predicted to hit Creb1 as a target, and binding sites for CREB lie upstream of miR-96, 182, 183, 200a, b, c, 220a*, and 200b*. This suggests that a similar feedback loop arrangement may also exist for CREB as well, similar to what has been described for other CREB-stimulated miRNAs and target genes (Wu and Xie, 2006). Because these miRNAs are down regulated in NLH rats, but not LH rats, this can be interpreted as a homeostatic response intended to minimize repressive effects on Creb1. Besides characterizing miRNAs that showed changes in mean expression levels across treatment groups, a large core coexpression module was identified, consisting of miRNAs that are strongly correlated with each other across individuals of the LH group, but not either the NLH or tested control group. The presence of such a module implies that the normal homeostatic miRNA response to repeated inescapable shock is not merely absent or blunted in LH rats; rather, gene expression networks are actively reorganized in LH rats, which may support their distinctive persistent phenotype. The set of miRNAs included in the core module may be indicative of factors that maintain the LH phenotype independent of the stress response per se. In conclusion, this study indicates that miRNAs participate in the

alterations of gene expression networks that underlie the normal (NLH) and aberrant (LH) response to repeated shocks.

5.4. microRNAs in affective illnesses

A direct study of miRNAs in depressed patients is lacking; however, my laboratory has recently conducted a preliminary study in which global expression patterns of miRNAs in the prefrontal cortex of drug-free depressed patients was examined (Dwivedi, Smalheiser, unpublished data). It was found that 21 miRNAs were significant down-regulated in the prefrontal cortex of depressed subjects compared with normal controls. Seven of the affected miRNAs shared overlapping 5'-seed sequences that are involved in target recognition. An analysis of predicted mRNA targets suggested that these miRNAs regulate an overlapping set of biological targets, including brain-active transcription factors, NOVA1, signaling proteins, and genes involved in neurotransmitter release and synaptic plasticity. Also, miRNA pathways were not simply down regulated in the depressed group but were also dramatically reorganized in a coordinated manner. A set of 29 miRNAs was identified; their expression was not correlated at all with each other in the control group but formed a highly correlated network in the depressed group. Most of these miRNAs showed no significant change in mean expression levels across groups. Such a network is likely to reflect the influence of shared factors that regulate the miRNAs in depressed subjects (eg, transcription factors or proteins involved in the miRNA processing pathway that regulate specific subsets of miRNAs). Some of the predicted targets for altered miRNAs are VEGF receptor Neuropilin-1, GAP43, SNAP25, synaptotagmin-1, synaptobrevin-1, and Lim kinase-1. Four different down-regulated miRNAs all targeted the same validated growth factor, VEGFA (miR-20b, 20a, 34a, 34b*). As mentioned earlier, VEGFA is a growth factor implicated in depression in both humans and animal models. Vascular endothelial growth factor treatment up regulates nuclear expression of E2F1, another validated target, among other cell cycle-related genes. Other validated targets include Bcl2 (miR-34a), DNMT3B (miR-148b), and MYCN (miR-101, 34a). In addition, a variety of signaling proteins, ion channels, and ubiquitin ligases were also identified. Several of these predicted targets have been linked to affective disorders.

Very recently, Xu et al. (2010) reported examined the relationship between ss178077483 polymorphism in the miRNA-30e precursor in 1088 depressed patients and 1102 control subjects in the Chinese population. They also determined the effects of miR-30e ss178077483 on the development of P300 event-related potential components induced by an auditory odd-ball task. P300 latency was associated with miR-30e ss178077483 genotypes and there was statistically significant positive association between miR-30e ss178077483 and depressed subjects. Individuals with the C/T genotype had a longer P300 latency than those carrying the C/C genotype. This study suggests that miRNA polymorphisms may play an important role in depressive susceptibility and that miRNAs may be involved in the etiology of major depression.

In bipolar patients, there have been some direct studies demonstrating that miRNAs may play an important role in this disorder. In the prefrontal cortex of bipolar patients, Kim et al. (2010a) examined global expression of miRNAs and found that expression levels of 15 miRNAs are altered. Of these, miR-504, miR-145, miR-145*, miR-22*, miR-133B, miR-154*, and miR-889 were up regulated and miR-104-3p, miR-29a, miR-32, miR-874, miR-454*, and miR-520c-3p were down regulated. Target analysis for 4 validated miRNAs yielded prediction of 2666 genes; several of them were from networks related to nervous system function and disease.

Because miR-134 has been shown to regulate brain development and dendritic spine morphology, Rong et al. (2010) examined the expression of this miRNA in the peripheral

cells of 21 drug-free patients with bipolar I manic illness and 21 matched healthy controls. They also followed up this study 2 and 4 weeks after drug treatment to these patients with bipolar manic illness. They found that the expression of miR-134 was significantly decreased in bipolar patients compared with healthy controls. On the other hand, the level miR-134 increased 2 and 4 weeks after drug treatment compared with drug-free bipolar patients. These results suggest that drug treatment was effective in increasing the level of miR-134 in bipolar patients. There was also a negative correlation between plasma miR-134 level and Beck-Rafaelsen Manic scale scores before and after treatment, suggesting that a decreased level of miR-134 may be associated with the pathophysiology and severity of manic symptoms in bipolar patients.

In an important study, Zhou et al. (2009) examined the effects of long-term lithium and valproate treatment on the expression of various miRNAs in rat hippocampus. They found that expression levels of 37 miRNAs were altered by lithium, whereas 31 miRNAs were altered by valproate. There were 9 miRNAs that were regulated by both lithium and valproate. These include let-7b, let-7c, miR-105, miR-128a, miR-24a, miR-30c, miR-34a, miR-221 (downregulated), and miR-144 (upregulated). The most significant signaling pathways that are targeted by these miRNAs are the PKC, PTEN, ERK-MAP kinase, Wnt/ β -catenin, and β -adrenergic signaling pathways. Some of these pathways have been shown to be altered in depressed and bipolar disorder (reviewed in Machado-Vieira et al., 2010). Also, the downregulation of miR-128a, miR-24, and miR-34a by chronic treatment with lithium or valproate significantly up regulated hippocampal protein levels of DPP10, GRM7, and THRB. Interestingly, GRM7 has emerged as a novel candidate gene for bipolar disorder (Saus et al., 2010).

The effect of lithium on the expression of miRNAs has also been studied in lymphoblastoid cell lines. Chen et al. (2009) examined the expression patterns of 13 miRNAs in 20 lymphoblastoid cell lines with or without lithium treatment in culture. They found that of 13 miRNAs, 7 miRNAs (miR-221, miR-152, miR-15a, miR-494, miR-155, miR-181c, and miR-34a) were altered by lithium. Of these 7 miRNAs, 4 (miR-34a, miR-152, miR-155, and miR-221) showed a consistent change after a longer treatment period (day 16). Target prediction analysis revealed 29 targets for miR-221 and 10 targets for miR-34a. These targets were inversely correlated with the expression of these two miRNAs. Some of the identified predicted targets were AP2A1, AP2S1, CD2AP, EIF-1, and VCL. These targets are involved in gene ontology-defined biological processes, such as macromolecular complex assembly, protein complex assembly, and cellular component assembly.

Interestingly, (Baudry et al., 2010), showed that 5-HT transporter is a target of miR-16, which has a higher expression pattern in noradrenergic neurons than that in serotonergic neurons. A reduction of miR-16 in noradrenergic neurons caused de novo 5-HT transporter expression, and long-term treatment with fluoxetine to mice increased miR-16 levels in serotonergic raphe nuclei, which, in turn, reduced 5-HT transporter expression. Furthermore, raphe responded to long-term fluoxetine treatment by releasing S100 β , which, in turn, acted on the noradrenergic neurons of the locus coeruleus. Thus, by lowering miR-16 levels, S100 β unlocked the expression of serotonergic functions in this noradrenergic brain area. This study suggests that miR-16 acts as a central effector in regulating 5-HT transporter expression and mediates the adaptive response of serotonergic and noradrenergic neurons to fluoxetine treatment (Baudry et al., 2010).

From the studies of human postmortem brain and peripheral cells, it is clear that the expression of miRNAs is altered in depression as well as in bipolar disorder patients. The results of studies examining the effect of psychoactive drugs on miRNAs also point in this direction.

5.5. microRNAs in schizophrenia

As compared to affective illnesses, the role of miRNAs in schizophrenia has been studied in greater detail. Since this review focuses mainly on depression, I will briefly summarize the findings of miRNAs in schizophrenia. Beveridge et al. (2008) analyzed global miRNA expression in the superior temporal gyrus of schizophrenia patients and reported that expression of miR-181b is upregulated. The predicted targets of this microRNA are calcium sensor gene visinin-like 1 (VSNL1) and the ionotropic AMPA glutamate receptor subunit (GRIA2), which have been shown to be down-regulated in the same cortical tissue from the schizophrenia group. This group of investigators further studied miRNA expression in Brodmann area 46 of schizophrenia patients and found that 25 miRNA were significantly upregulated in and 3 miRNA were significantly downregulated (Santarelli et al., 2011). They also examined the miRNA biogenesis machinery in this brain area of schizophrenia subjects. The expression of Dicer was increased by 1.23-fold. The DGCR8 and Drosha also displayed a robust 1.5-fold increase, however, they were not statistically significant. Similar findings schizophrenia-associated increase in global miRNA expression was reported in superior temporal gyrus and the dorsolateral prefrontal cortex, which was associated with an elevation of primary miRNA processing and corresponded with an increase in the microprocessor component DGCR8 (Beveridge et al., 2010). Individuals with 22q11.2 microdeletions have cognitive and behavioral impairments and the highest known genetic risk for developing schizophrenia. Since DGCR8 gene is disrupted by the 22q11.2 microdeletion, Fénelon et al. (2011) determined the effect of DGCR8 deficiency on the structure and function of cortical circuits and intrinsic and synaptic properties of layer 5 pyramidal neurons in the prefrontal cortex of DGCR8 (+/-) mutant mice. They showed that heterozygous DGCR8 mutant mice had fewer cortical layer 2/4 neurons and basal dendrites of layer 5 pyramidal neurons had slightly smaller spines. In addition, these mice had greater short-term synaptic depression. Their findings suggest altered short-term plasticity as a neural substrate underlying the cognitive dysfunction and the increased risk for schizophrenia associated with the 22q11.2 microdeletions.

In another postmortem brain study, Kim et al. (2010a) found significant alterations in the expression of several miRNAs in prefrontal cortex of schizophrenia subjects. These include miR-34a, -132, -132*, -212, -544, -7, and 154*. Several predicted target genes regulated by these microRNAs include AKT1, *alpha synuclein*, *GRIN1*, *GRIN2A*, and *GRIN2B* (encode different NMDA receptors), *BDNF*, and *Dopamine receptor 1*, all of them have been shown to be involved in schizophrenia pathology. In this respect, using a postmortem case-control design, Mellios et al. (2008) examined the association between BDNF protein, NPY/SST/PV mRNAs, and two BDNF-regulating miRNAs (miR-195 and miR-30a-5p) in prefrontal cortex of schizophrenia subjects. They found deficits in NPY and PV mRNAs. BDNF protein levels showed strong positive correlations with NPY and SST and a robust inverse association with miR-195 levels, suggesting that prefrontal deficits in a subset of GABAergic mRNAs are dependent on the regional supply of BDNF, which in turn is regulated via miRNA-mediated mechanism.

Zhu et al. (2009) performed analysis of all known human miRNAs by comparing their frequencies of targeting schizophrenia-associated genes to the targeting frequencies for all known human genes. They also identified the miRNAs predicted to target putative schizophrenia genes with the highest frequencies. They found 10 miRNAs (miR-181a, -769, -768, -299, -30a, -515, -369, 346, 566, 199a), with the highest predicted targeting frequencies; most of them target the 3'-UTRs of schizophrenia genes with frequencies similar to those expected based on a random cross section of human genes. Of them, miR-566 and miR-346, targeted genes from schizophrenia gene more frequently. Interestingly, the miR-346 gene is located in the intron 2 of the GRID1 gene, proposed to be critical in schizophrenia susceptibility.

Feng et al. (2009) tested the hypothesis that ultra-rare mutations in miRNA collectively contribute to the risk of schizophrenia. For this, they amplified and sequenced 59 miRNA genes on the X-chromosome of males with and without schizophrenia spectrum disorders. Eight ultra-rare variants in the precursor or mature miRNA were identified in eight distinct miRNA genes in 4% of analyzed males with schizophrenia. Functional analyses of ectopically expressed copies of the variant miRNA precursors demonstrated loss of function, gain of function or altered expression levels.

6. Other microRNA targets relevant to affective illnesses

An excellent review on the aspect of miRNA regulation of serotonergic transmission has recently been published (Millan, 2011). As mentioned earlier, fluoxetine regulates expression of miR-16, which acts as a negative regulator of serotonin transporter by binding to the 3'-UTR region of serotonin transporter mRNA (Baudry et al., 2010). miR-15 and miR-16 repress translation of Bcl-2 (Cimmino et al., 2005), an anti-apoptotic protein, which has been shown to play a role in affective illnesses and the mechanisms of action of mood stabilizers (Marmol, 2008; Kim et al., 2010b). miR-124, which possesses a putative CREB binding site in its promoter region, regulates the expression of CREB, a key transcription factor implicated in depression (Dwivedi et al., 2003a). The expression of miR-124 in turn is regulated by serotonin (Rajasethupathy et al., 2009). Recently, Jensen et al. (2009) identified an element (A-element) within serotonin receptor 1B mRNA that confers repression by miR-96. The repressive activity of this element was attenuated by a common human variant (G-element) that disrupted a nucleotide critical for its interaction with miR-96. They found that individuals homozygous for the A-element report more aggression related conduct-disorder behaviors compared to individuals with one or two copies of the G-element. Interestingly, 5-HT_{2A} receptors, strongly implicated in depression and suicide, is repressed by miR-195, miR-15a, and miR15b (Beveridge et al., 2010). Although not implicated in affective illnesses, it has been shown that another serotonin receptor, 5HT4 is repressed by miR-195, which also appears to regulate the translation of BDNF and glutamate receptors (Guo et al., 2010).

7. Conclusion and Future Studies

From the evidence discussed in this review article, it appears that miRNAs play pivotal roles in many biological phenomena, including synaptic plasticity, neurogenesis, and stress responses. All of these have been shown to participate in affective illnesses. Studies in animal models of depression and in human postmortem brain are rapidly evolving, providing direct evidence that expression levels of miRNAs are altered in depressed and bipolar patients. In addition, psychoactive drugs modify the expression of some of the miRNAs that are altered during stress or in brain of behaviorally depressed rats, not only substantiating these findings, but also suggesting that mechanisms of action of psychoactive drugs may in part be mediated via miRNAs. It is interesting to note that miRNAs are also altered in the blood cells of patients with affective illnesses. This provides an excellent opportunity for screening peripheral miRNAs, which can eventually be used for diagnosis and treatment response, as has been demonstrated in various types of cancer (Varol et al., 2011). Much work, however, is needed in this direction since miRNAs may be expressed differently in various blood cell types. In addition, whether miRNAs in a specific blood cell type truly represent brain-derived miRNAs, is unclear at the present time.

As previously discussed, local control of protein synthesis near synapses is critical for normal learning and dendrite spine morphogenesis. Because both dicer and pre-miRs are expressed in synaptic fractions and are strongly associated with postsynaptic densities, at least some mature miRNAs are locally processed near synapses. It will be interesting to

examine how these synaptic miRNAs play a role in pathophysiological conditions. There is a possibility that miRNAs may regulate homeostasis of neural pathways, particularly synaptic pathways, that have been involved in depression (e.g., CREB-BDNF pathways that are involved in a variety of feedback and feed-forward networks involving miRNAs) rather than simply inhibiting expression of individual targets. In addition, much work is required at some additional levels. For example, the expression patterns of miRNAs vary from one brain region to another and their expression is cell type specific. Thus, further studies are needed to characterize the cell type and layer distribution of these miRNAs within specific brain regions.

Mature miRNAs are effector molecules that directly regulate target mRNAs, so measuring these should have a relatively direct relationship to function. However, even if changes are observed in specific miRNAs and are statistically significant, understanding the biological interpretation of these changes will require much additional work. miRNAs can be regulated at multiple levels: transcription of primary miRNA precursor transcripts, processing by drosha into miRNA hairpin precursors, transport out of the nucleus, processing by dicer into mature miRNAs, and incorporation into RISC. Furthermore, the presence of a given miRNA does not automatically ensure that it is functional. This depends on the levels of the miRNA, its location, the presence of a target mRNA, possibly other protein and RNA co-factors binding to the target mRNA, and possibly cellular activation events such as raised calcium levels or proteolytic cleavage or phosphorylation of miRNA pathway protein components.

It is well-known that if one detects changes in specific mRNAs by gene expression profiling, the finding needs to be independently validated by, e.g. PCR and sequencing. This caution applies to miRNAs as well, and is especially important since miRNAs often form families of closely related sequences that may cross-react on the arrays, especially if they differ by only one nucleotide. As well, sequencing is important because polymorphisms in the miRNA sequence occurring in different individuals may cause altered binding to the arrays that will appear as altered levels of expression. In cases where it is difficult to distinguish closely related miRNAs by PCR and sequencing alone, one needs to identify the specific miRNA precursors that are expressed in the brain region. Also important is to examine the potential reasons for altered miRNA expression. Is it because of 1) genetic changes in the promoter region upstream of primary miRNA gene transcripts, the pre-miRNA hairpin, or the mature miRNA; 2) RNA editing of transcripts or epigenetic suppression of the chromosomal region encoding the miRNAs; or 3) changes in the miRNA machinery (e.g., dicer, drosha, or RISC)? Another important aspect is to confirm the predicted functional targets of depression-associated miRNAs. A promising approach would be to knock down or overexpress specific depression-related miRNAs and examine the behavioral phenotype.

Hybridization-based microarray platforms recognize miRNAs robustly despite minor variations and end-modifications, but may have difficulty distinguishing closely related miRNAs belonging to the same family. RT-PCR based miRNA analyses are extremely sensitive, specific and suited for quantitation, but the PCR primers are based on canonical miRBase sequences and may not detect all forms of the miRNAs that are actually expressed in a given tissue. Deep sequencing is not particularly suited for quantitation, and it is possible that some miRNAs or other small RNAs may not be detected (e.g., if their end-modifications are not amenable to adaptor addition). However, deep sequencing can provide detailed information that is not possible using other available platforms: alternate dicer processing of pre-miRs, “nibbling” and nontemplated nucleotides added to mature miRNAs, RNA editing, polymorphisms, offset and antisense miRNAs, minor miRNA species arising from the opposite arm of the pre-miR, etc. These variations may entirely alter miRNA processing and stability, and more importantly, may completely change the spectrum of targets that they regulate. Deep sequencing data can also be analyzed to identify sequences

that do not correspond to annotated miRNAs yet show significant changes in expression in a given psychiatric illness. This may provide a potential source of sequence data for future analyses of novel and atypical miRNAs, endogenous small interfering RNAs, or other small RNAs.

A search for risk variants by conducting genome-wide association studies is beginning to identify novel ncRNAs as candidate disease-associated genes. Most recently, several polymorphisms in post-transcriptional regulatory elements related to miRNAs have been identified. The deep sequencing of non-coding RNAs along with disease variants via genomic sequencing will be critical in identifying the role of non-coding RNAs to disease pathophysiology (Sethupathy and Collins, 2008).

As is well known, besides miRNAs, there are several other non-coding RNAs have been identified. These include snRNAs, snoRNAs, siRNAs, and piRNA (reviewed in Hüttenhofer et al., 2005; Taft et al., 2010). siRNAs and piRNAs have been shown to participate in epigenetic regulation by directing chromatin remodeling events (Verdel et al., 2009). On the other hand, snoRNAs act as guides for the modification of other RNA molecules such as rRNAs. In addition numerous long non-coding RNAs have been detected that consist of several thousand nucleotides or more. These RNAs are transcribed from Polymerase II promoters, and are alternatively spliced and/or polyadenylated. Though, the primary roles of these long non-coding RNAs appears to be as epigenetic regulators of protein-coding gene expression, they have also been shown to be regulated by transcription factors, such as CREB, Sp1, c-myc, Sox2, NF- κ B and p53. The roles of these non-coding RNAs are evolving rapidly and as their role in cognitive functions has recently been discovered (Qureshi and Mehler, 2011). Thus, it will be interesting to examine these non-coding RNAs in neuropsychiatric disorders. One example is that mutant mice lacking *Evf2* show reduced numbers of GABAergic interneurons early in development and reduced synaptic inhibition in adulthood (Vangompel et al., 2009).

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Figure 1.

Biogenesis of miRNAs. miRNAs are transcribed by RNA polymerase II to generate primary microRNA (pri-miRNA). The Drosha/DGCR8 catalyzes the formation of precursor miRNA (pre-miRNA). pre-miRNA is then exported to cytoplasm by Exportin5 in conjunction with RanGTP. In the cytoplasm, pre-miRNA is cleaved into miRNA:miRNA* duplex by Dicer/ Tar RNA-binding protein or PKR activating protein (PACT). One of these 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. The RISC complex binds to specific “short-seed” sequences located predominantly within the 3-UTR region of mRNAs and can interfere with translation of the mRNA and/or may reduce mRNA levels.

Table 1
microRNAs and their possible association with depression and related disorders

microRNA	Target	Known effect/clinical relevance	Reference
miR-124	Sox9	Neuron formation, neurogenesis	Chen et al., 2009
miR-137	Ezh2, a histone methyl transferase	Modulate proliferation and differentiation of adult neural stem cells, neurogenesis	Szulwach et al., 2010
miR-280, miR-289	CaMKIIa	Long-term potentiation, synaptic protein synthesis, synaptic plasticity	Ashraf et al., 2006
miR-134	LiM kinase I	Dendritic spine growth, synaptic plasticity, bipolar disorder	Schratt et al., 2004; 2006; Rong et al., 2010
miR-138	Acyl protein thioesterase 1	Palmitoylation of proteins, known to function at synapse, spine enlargement, synaptic plasticity	
miR-132, miR-124	CREB, SERT1, NR2A, NR2B, GlutRI	LTP, dendritic morphogenesis, neurite outgrowth, synaptic plasticity, oxidative stress, circadian rhythms	Vo et al., 2005; Wayman et al., 2008; Rajasekharan et al., 2009; Gao et al., 2010; Impey et al., 2010; Kawashima et al., 2010
miR-30a, miR-195	BDNF	Neurite outgrowth, synaptic functions, synaptic plasticity, stress, major depression, antidepressant function	Mellios et al., 2008
miR-18a, miR-124a	GR	Inhibit translation of glucocorticoid receptor, stress-related disorders, major depression	Uchida et al., 2008; Vreugdenhil et al., 2009
miR-9, miR-9*, miR-26b, miR-29b, miR-30b, miR-30c, miR-30e, miR-125a, miR-126-3p, miR-129-3p, miR-207, miR-212, miR-351, miR-423, miR-487b, miR-494, miR-690, miR-691, miR-709, miR-711, and Let-7 a-e		Increased during acute restrained stress	Rinaldi et al., 2010
miR-134, miR-183, miR-132, Let-7a-1, miR-9-1, and miR-124a-1	LiM kinase I, CREB, splicing factors, SC35, SRP46, and SFRS11	Synaptic plasticity, cholinergic neurotransmission, altered during acute or chronic immobilization stress	Meerson et al., 2009
pre-miRNAs 132, 124-1, 9-1, 9-3, 212, 29a	Repressor element-1 silencing transcription factor (REST) 4 controls expression of these miRNAs	Dendritic length, neuronal activity, synaptic density, increased in frontal cortex during maternal separation-induced stressed mice, increase risk of depression in adulthood	Uchida et al., 2010
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	Zeb1, Zeb2; CREB; binding sites for CREB lie upstream of miR-96, 182, 183, 200a, b, c, 220a*, and 200b*	Most enriched in synaptic fractions, down regulated in NLH rats relative to tested controls; showed a blunted response in LH rats. All encoded at a few shared polycistronic loci, suggesting that their down regulation is coordinately controlled at the level of transcription.	Smalheiser et al., 2011
miR-20b, 20a, 34a, 34b*, miR-148b, miR-101	VEGFA, Bel2, DNMT3B, MYCN	Growth factor, epigenetic modulation, altered in prefrontal cortex of depressed subjects	Dwivedi, Samlheiser (unpublished observation)
miR-16	SERT	Fluoxetine decreases SERT expression via miR-16	Baudry et al., 2010
miR-545	SERT		Jensen et al., 2009
miR-96	5HT1B	Conduct disorder	

microRNA	Target	Known effect/clinical relevance	Reference
miR-196	5HT4		Guo et al., 2010
miR-195, miR-15A, miR-15b	5HT2A		Beveridge et al., 2010