Published in final edited form as:
Pharmacol Ther. 2011 April ; 130(1): 26–37. doi:10.1016/j.pharmthera.2011.01.004.

MicroRNAs: Meta-controllers of gene expression in synaptic activity emerge as genetic and diagnostic markers of human disease

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Abstract

MicroRNAs are members of the non-protein-coding family of RNAs. They serve as regulators of gene expression by modulating the translation and/or stability of messenger RNA targets. The discovery of microRNAs has revolutionized the field of cell biology, and has permanently altered the prevailing view of a linear relationship between gene and protein expression. The increased complexity of gene regulation is both exciting and daunting, as emerging evidence supports a pervasive role for microRNAs in virtually every cellular process. This review briefly describes microRNA processing and formation of RNA-induced silencing complexes, with a focus on the role of RNA binding proteins in this process. We also discuss mechanisms for microRNA-mediated regulation of translation, particularly in dendritic spine formation and function, and the role of microRNAs in synaptic plasticity. We then discuss the evidence for altered microRNA function in cognitive brain disorders, and the effect of gene mutations revealed by single nucleotide polymorphism analysis on altered microRNA function and human disease. Further, we present evidence that altered microRNA expression in circulating fluids such as plasma/serum can correlate with, and serve as, novel diagnostic biomarkers of human disease.

Keywords

MicroRNA; 3′ untranslated region; brain disorders; dendrite; diagnostic marker; eukaryotic initiation factor; fragile X mental retardation protein; human disease; mRNA stability; plasticity; RNA binding protein; RNA-induced silencing complex; single nucleotide polymorphism; translation

1. Introduction

The discovery of microRNAs at the turn of the 21st century marked the beginning of a new era in cell biology, and permanently changed our view of the relationship between gene

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Conflict of Interest S. Ceman and J. Saugstad have no conflicts of interest to declare.

expression and protein expression. Analysis of the correlation between genes encoding messenger RNAs (mRNAs) and human disease has expanded to include those sequences in the remaining ~90% of eukaryotic genomes that generate non-coding RNAs. The microRNAs serve as meta-controllers of gene expression and are crucial for the cellular changes that are necessary for development. In the brain, microRNAs serve essential roles in dendritic spine formation and function, and in synaptic plasticity are required for normal cognitive function. A complete understanding of the mechanisms that regulate microRNA expression is critical, especially given the correlation between dysregulated microRNAs and several neurological disorders (Saugstad, 2010). Although not discussed herein, recent reviews discuss two very important levels of microRNA regulation including new transcription of microRNA-encoding genes by activity-regulated transcription factors like CREB and Mef (Flavell et al., 2008), and regulation of RNA processing events that lead to mature microRNAs (Newman et al., 2010).

There is intense interest in microRNA gene mutations as the underlying cause for human diseases, and for their potential as novel therapeutics for the treatment of such disorders. The probability that mutations in genes that encode microRNAs, components of the biosynthesis machinery, and/or their mRNA targets, drives new genetic studies to identify links to human diseases. The analysis of single nucleotide polymorphisms provides a powerful tool to link altered microRNA expression with human diseases. MicroRNAs are also found in circulating fluids such as plasma/serum and cerebrospinal fluid (CSF), and studies show evidence for altered expression of microRNAs correlating with human diseases. Thus, the microRNAs have evolved from their role as metacontrollers of gene expression to serve as genetic markers and non-invasive biomarkers for the diagnosis, treatment and progression of human disease.

2. MicroRNA processing, RISC formation and RNA binding proteins

MicroRNAs are small, ~20-24 nucleotide, genomically encoded RNAs that regulate mRNA expression by base-pairing to sequences in the mRNA, usually in the 3′ untranslated region (3′UTR) (Bartel, 2009). Primary precursor microRNAs are transcribed by RNA polymerase II in the nucleus and are then processed into 70-80 nucleotide precursor microRNAs by the microprocessor complex, minimally composed of the ribonuclease (RNase) III endonuclease Drosha and its binding partner DGCR8/Pasha (Lee et al., 2003; Newman et al., 2010). Precursor microRNAs are exported to the cytoplasm by exportin 5 (Yi et al., 2003) where they are processed again into short ~22 nucleotide duplexes by the RNase III Dicer, which is part of a pre-microRNA processing complex (Bartel, 2004; Lee et al., 2003). The pre-microRNA, Dicer and another RNase III endonuclease, Argonaute 2 (Ago2), form the RNA-induced silencing complex (RISC) (Landthaler et al., 2008). One strand of the short RNA duplex is loaded into the Ago2-containing, RISC (Newman et al., 2010) to form a microRNA silencing complex, while its complementary (passenger) strand is subsequently degraded (Filipowicz et al., 2005). In mammals, Ago2 is the only Argonaute family member with endonuclease activity (Filipowicz et al., 2005; Hock et al., 2008). Analysis of the mRNAs that co-immunoprecipitate with Dicer suggests that Dicer and its associated proteins are not part of the microRNA effector complexes, since the Dicer-enriched transcripts share no significant similarity to the microRNA targets immunoprecipitated with Ago (Landthaler et al., 2008). Thus, the Ago proteins disengage from the RISC loading complex before targeting mRNAs (MacRae et al., 2008). Nucleotides in the mature microRNA form base-pairs with complementary sequences in the mRNA which forms a short helix. Complete complementarity between the 20-22 nt length of the small RNA and the mRNA leads to degradation, hence ‘silencing’ of the transcript. In contrast, partial base pairing, specifically between nucleotides 2-8 of the microRNA (its seed sequence) and the mRNA leads primarily to translation suppression and some degradation (Baek et al., 2008).

A major unresolved question in microRNA-mediated translation regulation is how do the microRNAs physically find their target mRNAs in the cell, and how do protein-protein interactions facilitate targeting (Nelson et al., 2004)? The free energy of base pairing drives association of the complementary microRNA with its target mRNA (Hofacker, 2007); however, it is not known how these two nucleic acid strands are brought into close enough proximity to achieve helix formation. As described above, mature microRNAs are associated with Ago2. A number of proteins have been co-purified with Ago2 that could potentially participate in this interaction, including family member Ago1, several RNA binding proteins such as the Fragile X mental retardation protein (FMRP), and putative RNA binding proteins like Vasa intronic gene (VIG), and an RNA recognition motif-containing protein, Trinucleotide repeat-containing 6B, (TNRC6B) (Caudy et al., 2002; Ishizuka et al., 2002; Meister et al., 2005). Both FMRP and TNCR family members will be discussed in more detail below, along with other proteins that associate with Ago2 or that effect microRNA-mediated regulation, including the Pumilio proteins and the putative helicase MOV10.

FMRP is an RNA binding protein that is absent in the most common form of inherited mental retardation, Fragile X Syndrome (Bassell et al., 2008). In addition to associating with approximately 4% of brain mRNAs (Ashley et al., 1993; Brown et al., 2001), FMRP has also been implicated in translation regulation—both activating and suppressing translation of its bound RNAs (Brown et al., 2001). Two separate studies in *Drosophila* showed that FMRP associated with Ago2 (Caudy et al., 2002; Ishizuka et al., 2002). A subsequent study showed that mammalian FMRP and its autosomal paralogs, fragile X-related proteins 1 and 2 (FXR1P and FXR2P) associated with Dicer and other components of RISC including Ago (Jin et al., 2004). Phosphorylation of FMRP eliminates association with Dicer and may function as a switch for association with the microRNA pathway (Cheever et al., 2009). FMRP has been demonstrated to directly associate with microRNAs *in vitro* and to specifically anneal microRNAs to RNAs containing the correct seed sequence (Plante et al., 2006). The *Drosophila* ortholog of FMRP has also been shown to regulate levels of miR-124, a microRNA that regulates dendritic branching (Xu et al., 2008). Importantly, FMRP isolated from brain specifically associates with a collection of microRNAs including miR-134 and miR-125b, the latter regulating NR2A receptor expression in an FMRP-dependent manner (Edbauer et al., 2010) (Figure 1). Thus, FMRP is an RNA binding protein that directly or indirectly associates with microRNAs to regulate protein expression of FMRP-bound mRNAs.

TNRC6B is one member of a family of proteins that are vertebrate paralogs of the scaffolding protein, GW182. GW182 is present in the core microRNA silencing complex and is important for localization to processing bodies (P-bodies), as well as for translation silencing and mRNA degradation (Behm-Ansmant et al., 2006; Eulalio et al., 2008). All family members contain an abnormally high content of GW/WG repeats and a C-terminal RNA recognition motif domain (Baillat et al., 2009). TNRC6A, -B and -C associate with all four human Ago proteins complexed with microRNAs, and the TNRC6 proteins contain a P-body localization domain (Baillat et al., 2009). GW182 is required for microRNA-mediated silencing, although it is unclear how these proteins perform that function. Recent evidence suggests that GW182 interferes with mRNA circularization and also recruits the deadenylase complex through interaction with poly(A) binding protein C1 (Zekri et al., 2009).

Pumilio proteins are examples of RNA binding proteins that enhance microRNA modulation. Human Pumilio proteins PUM1 and PUM2 are members of the PUmilio-Fem-3 binding factor (PUF) family defined by the presence of an RNA binding domain that consists of eight repeats, each of which makes contact with a different RNA base (Edwards et al., 2001; Gupta et al., 2008; Wang et al., 2002). To identify the specific RNAs

recognized by PUM1 and 2, Galgano and colleagues queried microarrays with RNAs co-immunoprecipitated with PUM1 and 2, and identified more than one thousand PUM1-associated mRNAs and hundreds of PUM2-associated mRNAs (Galgano et al., 2008). These results suggest that PUM proteins could potentially regulate up to ~15% of the transcriptome of the cell. Similar to the mRNA targets of yeast and *Drosophila* Pumilio proteins, most of the human PUM targets contain a characteristic PUF-binding motif in the 3′UTR. In addition, the PUF motif is enriched around predicted microRNA binding sites. A PUF protein is required for let-7 suppression of the *Caenorhabditis elegans* hunchback homolog 1 transcript (Nolde et al., 2007), suggesting that PUFs and microRNAs cooperate to suppress common targets (Galgano et al., 2008). Recently, human PUM2 was shown to participate in dendrite morphogenesis, synapse function and translational control, in part through association with the eukaryotic initiation factor (eIF) 4e mRNA (Vessey et al., 2010). A mechanism for how the Pumilio proteins associate with mRNAs to enhance miRNA-mediated silencing was recently elucidated in a study of the regulation of tumor suppressor protein p27. PUM1 was shown to bind the 3′ UTR of p27 at a site that forms a stem-loop structure with the miR-221 binding site. Binding of PUM1 favors opening of the structure, allowing increased access to the miR-221 and 222 binding sites (Kedde et al., 2010). The interplay between RNA binding proteins and microRNA-bound RISCs is more comprehensively covered in the recent review (Krol et al., 2010).

In addition to RNA binding proteins, the putative helicase MOV10 was also identified in Ago-containing complexes (Meister et al., 2005). Both MOV10 and TNRC6B were present in processing bodies (P-bodies) and were functionally required to mediate microRNA-guided mRNA cleavage of a reporter RNA (Chendrimada et al., 2007; Meister et al., 2005). Immunoprecipitated MOV10 did not contain Dicer activity, suggesting that MOV10 functions downstream of the Dicer cleavage step or that it only transiently associates with the RISC loading or assembly complex (Meister et al., 2005). MOV10 was first described as a putative RNA helicase in the characterization of a related protein MOV10L1 (Ueyama et al., 2003). MOV10 was likely called ‘putative’ because its nucleotide binding domain (amino acids DEAG) does not fit neatly into either the DEAD or DExD box family of RNA helicases (Mooslehner et al., 1991). Helicases are involved in all aspects of RNA metabolism including RNA processing and export, mRNA translation and decay (Abdelhaleem, 2010). Helicases may unwind duplex structures, or, less commonly, anneal nucleic acids (Pyle, 2008). In addition, helicases may function as protein displacement enzymes that facilitate the remodeling of large ribonucleoprotein complexes, as has been described for other RNA helicases, which include DEAD and DExH proteins (Jankowsky et al., 2000). Recently a role for MOV10 at the synapse was described (Banerjee et al., 2009). MOV10 is present in the synaptodendritic compartment in neurons and its degradation was triggered by activation of the NMDA receptor. Proteasome-mediated degradation of MOV10 led to an increase in expression of a subset of mRNAs, one of which encodes acyl-protein thioesterase 1 (APT1), a depalmitoylating enzyme that is regulated by miR-138. Palmitoylation modification is a mechanism utilized by many synaptic proteins that potentially regulate post-synaptic structures (Kang et al., 2008). Thus, degradation of MOV10 and possibly other components in the RISC may underlie the observation that both protein degradation and synthesis are required for synaptic plasticity (Ashraf et al., 2006; Fonseca et al., 2006).

Finally, some RNA binding proteins act to suppress microRNA mediated regulation. Examples include the evolutionarily conserved RNA-binding protein, dead end 1 (Dnd1), which binds conserved uridine-rich regions around microRNA binding sites to inhibit microRNA binding to their target sequences (Kedde et al., 2007). Another RNA binding protein that counteracts the activity of a microRNA is HuR (ELAV1) which binds an AU-

rich element to relieve CAT-1 mRNA from microRNA-122 mediated repression (Bhattacharyya et al., 2006).

3. MicroRNA regulation of translation and mRNA stability

The discovery of small RNA-mediated regulation of translation occurred in *Caenorhabditis elegans*, where lin-14 was the first gene shown to be regulated by a small RNA encoded by lin-4 (Lee et al., 1993). Lin-4 RNA was complementarity to multiple sites in the 3′UTR of the lin-14 gene, and lin-4 RNA suppressed expression of lin-14 protein with only a small effect on mRNA levels. Lin-14 was found on polyribosomes during translation suppression, suggesting that translation of lin-14 is blocked at a step after translation initiation, either by stalling ribosome elongation or by causing immediate proteolysis of the newly synthesized proteins (Olsen et al., 1999). In mammalian studies, small-RNA regulated transcripts were also found on polyribosomes (Gu et al., 2009; Maroney et al., 2006; Nottrott et al., 2006; Petersen et al., 2006). One group concluded that translation suppression might be mediated by co-translational peptide degradation (Nottrott et al., 2006), while another proposed that small RNAs impaired elongation (Gu et al., 2009). Using a reporter construct targeted by an exogenous small RNA, Petersen and colleagues concluded that repression by microRNAs is primarily due to ribosome drop-off during translation elongation (Petersen et al., 2006). Around the same time, a number of groups presented evidence for small RNA suppression of translation by blocking initiation. Pillai and colleagues showed that microRNAs inhibit translation by interfering with recognition of the RNA cap by eIF4E (Pillai et al., 2005). This work was followed by other studies that came to the same conclusion, i.e., that small RNAs blocked translation initiation, and not elongation, through association with eIFs, by mechanisms described in more detail below (Bhattacharyya et al., 2006; Chendrimada et al., 2007; Ding et al., 2009; Mathonnet et al., 2007).

Human RISC associates with proteins of the 60S ribosome and eIF6 (Chendrimada et al., 2007). eIF6 inhibits joining of the 60S and 40S ribosomal subunits to form the translationally competent 80S subunit (Ceci et al., 2003; Raychaudhuri et al., 1984; Russell et al., 1979; Valenzuela et al., 1982). Depletion of eIF6 specifically abrogates microRNA-mediated regulation of target protein and mRNA levels (Chendrimada et al., 2007). Thus, microRNA-directed complexes may use eIF6 to disrupt productive polysome formation and expose target mRNAs for degradation (Chendrimada et al., 2007). In contrast, Mathonnet and colleagues showed that microRNA-mediated inhibition was alleviated by providing an excess of the eIF4E complex, suggesting that sequestering of eIF4E participates in microRNA-mediated silencing (Mathonnet et al., 2007). Further, Kiriakidou and colleagues reported that Ago2 binds the 7-methylguanosine (m7G) cap of mRNA directly, supporting the notion that Ago2 and eIF4E compete for association with the mRNA cap structure to prevent translation initiation (Kiriakidou et al., 2007). For a more comprehensive review of this subject, see (Gu et al., 2010). Finally, as mentioned earlier, GW182 directly associates with Ago2 and is required for silencing. Two independent studies provided evidence that GW182 may disrupt association between eIF4G and PolyA binding protein, which is required for efficient translation initiation (Fabian et al., 2010; Zekri et al., 2009), providing still another mechanism for inhibition of translation initiation by RISC.

In addition to their usual role as translation suppressors, microRNAs have also been implicated in translation activation, particularly under conditions of cell stress. Under serum starvation, which induces cell cycle arrest, the AU-rich element of tumor necrosis factor mRNA enhances translation through association with Ago2 and FXR1P (Vasudevan et al., 2007). In a second study, Vasudevan and coworkers identified miR-369-3 as directing association of Ago2 and FXR1P to the AU-rich region to activate translation (Vasudevan et al., 2007). They further showed that let-7 and a synthetic microRNA, cxcr4, induced

translational up-regulation of target mRNAs upon cell-cycle arrest, suggesting that microRNA-protein complexes oscillate between repression and activation, changing based on the protein composition of the micro-ribonucleoproteins. Another example of a trans-activating microRNA is miR-10a, which was shown to activate translation of ribosomal proteins during amino acid starvation (Orom et al., 2008). Interestingly, translation activation occurs through a binding a site in the 5′UTR, while suppression is the consequence of miR-10a binding to a site in the 3′UTR (Orom et al., 2008). Similarly, liver microRNA-122 activates translation of Hepatitis C virus by binding sites in the 5′UTR (Henke et al., 2008).

This review would not be complete without addressing microRNA-mediated regulation of mRNA stability as a mechanism for reducing protein expression. MicroRNA-mediated mRNA destabilization is thought to occur when microRNA association with an mRNA leads to deadenylation, which promotes de-capping and more rapid degradation through standard RNA turnover processes (Behm-Ansmant et al., 2006; Eulalio et al., 2009; Giraldez et al., 2006; Wu et al., 2006). Recent studies suggest that microRNA-mediated mRNA destabilization may be more prevalent than originally thought (Baek et al., 2008; Guo et al., 2010). Although some mRNAs targeted by microRNAs are repressed without detectable changes in mRNA levels, mRNAs translationally repressed by more than a third also displayed detectable mRNA destabilization (Baek et al Nature 2008). For the most highly repressed mRNAs, destabilization usually comprised the major component of repression (Baek et al., 2008). Guo and colleagues sought to examine the means by which microRNAs regulate protein expression by using ribosome profiling to examine two different cell systems: over-expression of miR-1 and miR-155 which are not normally present in HeLa cells, or knockout of miR-223 in neutrophils. The translation state of the cellular mRNAs in both cell systems was examined by deep sequencing of ribosome-protected mRNA fragments (Guo et al., 2010). Although the authors found a statistically significant decrease in translation efficiency in the presence of the microRNAs, they found a much larger decrease in polyadenylated RNA, concluding that lowered mRNA levels account for most (~84%) of the decreased protein production (Guo et al., 2010). An independent study using arrays found a similar result (Hendrickson et al., 2009). Thus, microRNA-mediated regulation of protein expression levels includes both mRNA stability, as well as regulation at the level of translation.

4. MicroRNAs in dendritic spine formation and function, and synaptic plasticity

Most excitatory synapses in the mammalian brain are formed at dendritic protrusions called dendritic spines (Figure 1) (Bourne et al., 2008). Some neurons, such as hippocampal neurons, contain thousands of spines throughout the dendritic branches or arbors (Sorra et al., 2000). Dendritic spines have a distinct architecture, comprised of a base at the junction of the dendritic shaft, a constricted neck and then a bulbous head that contacts the axon (Hotulainen et al., 2009). These components can have various sizes, leading to three categories of dendritic spines based on their morphology: thin, filopodia-like protrusions (“thin spines”), short spines without a well-defined spine neck (“stubby spines”), and spines with a large bulbous head (“mushroom spines”) (Bourne et al., 2008). Importantly, spine structures are not static but change morphology based on neuronal activity and experience (Hotulainen et al., 2010). Functional and structural changes at spines and synapses are believed to be the basis of learning and memory (Kasai et al., 2010).

The actin cytoskeleton contributes to the overall structure of the spine. In addition, it anchors the postsynaptic receptors, facilitates trafficking of synaptic cargoes, and localizes the translation machinery (Hotulainen et al., 2010). Not surprisingly, defects in the

regulation of the actin cytoskeleton underlie many forms of cognitive impairment (Newey et al., 2005). MicroRNAs modulate dendritic morphology by regulating expression of proteins involved in the actin cytoskeleton (Impey et al., 2004; Loya et al., 2010; Schratt, 2009; Schratt et al., 2006; Siegel et al., 2009; Wayman et al., 2008), mRNA transport (Fiore et al., 2009; Tiruchinapalli et al., 2008) and neurotransmitter receptor levels (Edbauer et al., 2010; Karr et al., 2009; Simon et al., 2008). These mechanisms will be described in more detail below, as depicted in Figure 1.

Actin is the major cytoskeletal component of dendritic spines (Landis et al., 1983). The most likely role of actin in mature spines is to stabilize post-synaptic proteins and to modulate spine head structure in response to postsynaptic signaling (Hotulainen et al., 2010). Both the monomeric form of actin and filamentous polymers of actin are present in spines. The degree of actin polymerization affects dendritic spine morphology (Cingolani et al., 2008). Accordingly, maintenance of the actin monomer pool is essential for rapid reorganization of the actin cytoskeleton in response to stimulation. Actin-depolymerizing factors, also known as cofilins, induce depolymerization of actin (Hotulainen et al., 2005; Kiuchi et al., 2007). In neurons, cofilin1 is required for normal actin turnover and morphology of the dendritic spines (Hotulainen et al., 2009). LIM kinases (LIMK) phosphorylate and subsequently inactivate cofilins (Meng et al., 2002). Accordingly, inactivation of LIM kinase 1 (LIMK1) consistently inhibits the activity of cofilins, resulting in altered dendritic spine morphology and synaptic function (Meng et al., 2002). LIMK1 is regulated by the brain-specific microRNA miR-134 and is localized to the synapto-dendritic compartment in hippocampal neurons (Schratt et al., 2006). Schratt and colleagues propose that association of LIMK1 mRNA with miR-134 keeps the LIMK1 mRNA in a dormant state while it is being transported within dendrites to synaptic sites, and stimulation with brain derived neurotrophic factor (BDNF) leads to derepression of LIMK1 by altering the activity of translation regulators (Schratt et al., 2006).

A major point of control in the regulation of the actin cytoskeleton includes the small Rho GTPases, including Rho A and Rac (Figure 1). Rho A inhibits cofilin activity, leading to actin filament and spine stabilization (Hotulainen et al., 2010). miR-132 regulates neuronal morphogenesis in developing neurons by repressing translation of the Rho family GTPase-activating protein, p250GAP (Hotulainen et al., 2010). In addition, neuronal activity also represses p250GAP translation in a miR-132 dependent manner (Wayman et al., 2008). By suppressing p250GAP levels, miR132 expression results in prolonged localized increases in Rac activity, which ultimately leads to activity-dependent dendritic outgrowth (Impey et al., 2010;Wayman et al., 2008). It has also been reported that p250GAP may regulate spine morphology by inhibiting Rho A, another cofilin inhibitor, which results in actin filament and spine stabilization (Hotulainen et al., 2010).

miR-138 is highly enriched in brain, localizes within dendrites and suppresses the size of dendritic spines in rat hippocampal neurons (Siegel et al., 2009). miR-138 controls expression of APT1, an enzyme which regulates the palmitoylation status of synaptic proteins. One downstream target of APT1 is the alpha(13) subunit of G proteins (Ga_{13}) (Siegel et al., 2009), which is an activator of Rho downstream of G-protein coupled receptors (Kurose, 2003). A miR-138-mediated increase in Ga_{13} palmitoylation and membrane localization could result in elevated Rho activity, potentially triggering spine shrinkage.

In addition to contributing to the overall structure of the spine, the actin cytoskeleton anchors the postsynaptic receptors. The actin-signaling pathways in spines are regulated by many synaptic receptors such as the excitatory NMDA and AMPA-type glutamate receptors. NMDA receptors regulate actin cytoskeleton by directly binding to actin-binding or actin-

regulating proteins like calcium/calmodulin-dependent protein kinase II, alpha-actinin and myosin regulatory light chain. The NR2A subunit of NMDA receptor was recently shown to be regulated by miR-125b (Edbauer, 2010) (Figure 1).

RNA-binding proteins are present in dendrites and important for the regulation of mRNA transport, and/or the stability or translation of local mRNAs in response to activity, thus they play a key role in neuronal physiology. MicroRNAs regulate key transcription factors such as MYC, E2Fs and MYB (Lal et al., 2009; Xiao et al., 2007), but there are fewer reports of microRNA-mediated regulation of RNA binding proteins. One of these is Pumilio2, an RNA binding protein implicated in the control of dendrite morphogenesis in *Drosophila melanogaster* that associates with Ago, and is regulated by miR-134, a member of a family of mRNAs transcribed in response to neuronal stimulation (Fiore et al., 2009) (Figure 1). Pumilio2 is a translational repressor and a component of somatic and dendritic granules, particularly stress granules (Vessey et al., 2006). Since the Pumilio mRNA is also present in dendrites, it is possible that miR-134 functionally interacts with Pumilio2 mRNA to regulate its expression in dendrites, leading to the redistribution of the mRNAs bound by Pumilio2 including eIF4E, post-synaptic density 95 homologs and a voltage-gated sodium channel (Fiore et al., 2009). Interestingly, the Pumilio2 mRNA is also found in the FMRP complex of mRNAs isolated from brain (Brown et al., 2001).

Another example of an RNA binding protein that is regulated by microRNAs is HuR, a sequence-specific RNA binding protein that regulates translation and RNA turnover, and in turn influences the cellular response to stress, proliferative signals, immune triggers and developmental cues (Abdelmohsen et al., 2007; Cherry et al., 2006; Gorospe, 2003). Ubiquitously expressed, HuR is also present in dendrites where it associates with mRNAs important for synaptic plasticity, learning and memory (Tiruchinapalli et al., 2008), and is regulated by miR-519 (Abdelmohsen et al., 2008) (Figure 1). FXR1P is another example of an RNA binding protein that was recently shown to be regulated by microRNAs 92b, 363 and 367 (Cheever et al., 2010). FXR1P is present in neurons (Bakker et al., 2000; Brown et al., 2001) and has been shown to directly bind Pak1 (Say et al., 2010), which regulates actin cytoskeleton dynamics to modulate spine morphogenesis (Boda et al., 2008).

Components of the RISC machinery have also been implicated in long-term memory in *Drosophila*. Dicer processes local precursor microRNAs into mature microRNAs, and these are incorporated into functional RISCs within or near the dendritic spine which bind to available target mRNAs in the vicinity (Figure 1). These may repress protein translation under resting conditions, yet permit a phasic burst of translation to occur transiently following subsequent synaptic activity. Loaded RISCs that are not bound to local mRNAs may serve to bind and trap mRNAs that are being transported down dendrites. Thus, locally formed microRNAs may mark the location of previously activated synapses and perform a type of synaptic tagging and capture (Smalheiser, 2007).

Evidence for localized protein translation at the synapse arose from studies showing a dramatic increase in polyribosomes associated with dendritic spines during periods of synapse growth (Steward, 1983). Subsequent studies revealed that synapse-specific gene expression and intracellular transport and synaptic targeting of select mRNAs play a key role in activity-dependent synaptic modification, which is critical for long-lasting synaptic plasticity (Steward et al., 2001; Steward et al., 2002). Dendritic P-body-like structures are a new class of RNP-particles found in the soma and dendrites of mammalian neurons (Cougot et al., 2008). These structures show motorized movements along dendrites and relocalize to distant sites in response to synaptic activation, thus they are proposed to regulate local translation by storing repressed mRNA-protein complexes in unstimulated cells, and releasing them on synaptic activation (Cougot et al., 2008).

There is accumulating evidence that induction of long-term potentiation (LTP) and long-term depression (LTD), two well-studied forms of synaptic plasticity, involves microRNAs. Microarray analysis determined the temporal expression of 62 hippocampal microRNAs following induction of chemical LTP (C-LTP) and metabotropic glutamate receptor-dependent LTD (mGluR-LTD) (Park et al., 2009). These studies revealed that induction of C-LTP and mGluR-LTD altered the expression of most hippocampal microRNAs, and while most of these microRNAs followed a similar temporal expression profile, they displayed distinct expression dynamics. Further, many microRNAs were upregulated at specific times after induction, suggesting that C-LTP and mGluR-LTD elicited microRNA-mediated suppression of mRNA translation to prevent excess protein synthesis during synaptic plasticity (Park et al., 2009). Studies in *Aplysia californica* showed that miR-124 is exclusively expressed in presynaptic sensory-motor synapses and restricts serotonin-induced synaptic facilitation via the regulation of CREB (Rajasethupathy et al., 2009). Nicotine-induced expression of miR-140* in rodent cells repressed translation of dynamin 1, which has an essential role in synaptic endocytosis in the brain and is likely important for neural plasticity in nicotine addiction (Huang et al., 2009). Recent studies also revealed differential regulation of primary and mature microRNAs by mGluR and NMDA receptor signaling following LTP induction by high-frequency stimulation of the medial perforant pathway in the dentate gyrus of adult rats (Wibrand et al., 2010). Changes in the expression of mature microRNAs were evident 2 hours after induction of LTP. Up-regulation of miR-132 and -212, and down-regulation of miR-219, were confirmed by quantitative real-time PCR (qRT-PCR). *In situ* hybridization validated the up-regulation of the primary miR-132/212 cluster and showed restricted expression to the soma of dentate granule cells (Wibrand et al., 2010). The LTP-induced increases in primary and precursor miR-132/212 were transcription- and mGluR-dependent, while NMDA receptor activation selectively down-regulated mature miR-132, -212 and -219 levels (Wibrand et al., 2010).

5. Evidence for altered microRNA function in cognitive brain disorders

MicroRNAs individually regulate up to several hundred genes, and collectively may regulate as much as two-thirds of the transcriptome. Miller and colleagues reviewed recent evidence which supports a role for microRNA dysregulation in psychiatric and neurological disorders such as schizophrenia, bipolar disorder, and autism, reviewed in (Miller et al., 2010). Small changes in microRNA levels affect the expression of multiple genes within a biological network, suggesting that dysregulation of microRNAs could underlie the complex changes observed in psychiatric diseases (Miller et al., 2010). Down syndrome (Trisomy 21) is a genetically determined neurodevelopmental disorder that is the most common genetic cause of cognitive impairment and congenital heart defects in the human population. This disorder usually results from an extra copy of human chromosome 21 (Hsa21) and the syndrome is characterized by short stature, epicanthal (eyelid) folds, abnormal fingerprints and palm prints, heart defects, poor muscle tone (delay of neurological development) and mental retardation (delay of intellectual development). Hsa21 harbors five microRNA genes; miR-99a, let-7c, miR-125b-2, miR-155, and miR-802. Expression profiling revealed that these microRNAs are over-expressed in fetal brain and heart specimens from individuals with DS (Kuhn et al., 2008b; Kuhn et al., 2010; Malinge et al., 2009; Patterson, 2009; Pritchard et al., 2008; Sethupathy et al., 2007). Thus, trisomy 21 gene dosage and resultant over-expression of Hsa21-derived microRNAs likely result in decreased expression of specific target proteins which contribute to features of the neuronal and cardiac DS phenotype.

Autism spectrum disorders (ASD) are complex neurodevelopmental/neurobehavioral disorders with varying degrees of severity. The syndrome is characterized by abnormalities in reciprocal social interactions, language development and/or usage, and by restricted

interests and repetitive behaviors. Approximately 10 to 15% of ASD cases are linked to genetic loci, and idiopathic autism represents 80 to 90% of ASD cases. Linkage analysis identified at least 15 genes from different chromosomes as autism susceptibility loci (Yonan et al., 2003a; Yonan et al., 2003b). However, most of these loci do not show association with autism in other studies, which likely reflects the heterogeneous phenotype of the disorder. Recent studies of microRNA expression in lymphoblastoid cell lines grown from autism and control subjects revealed differential expression for 9 of 470 mature microRNAs in autism subjects, and potential microRNA targets included several autism susceptibility genes (Talebizadeh et al., 2008). Further studies profiling of microRNA expression in lymphoblastoid cell lines revealed differential expression of microRNAs in autistic twins, relative to case-control sibling pairs (Sarachana et al., 2010). Altered expression was confirmed for two microRNAs, miR-29b and -219-5p. Validation of their predicted targets, DNA-binding protein inhibitor ID-3 (ID3) and serine/threonine-protein kinase (PLK2), was confirmed by overexpression or knockdown of miR-29b and miR-219-5p, respectively. In addition, microRNA and mRNA expression levels showed an inverse relationship between discordant twins and case-control sibling pairs (Sarachana et al., 2010). Thus, dysregulated microRNA expression likely contributes to the pathophysiology of autism, and gene expression analysis of lymphoblastoid cell lines can be used to identify altered microRNAs and/or their gene targets in ASD.

6. Genetics analysis of microRNAs in human disease

The discovery of microRNAs has increased the potential for genomic alterations in microRNA genes to serve as the underlying basis for many diseases. Single nucleotide polymorphisms (SNPs) could affect transcription of the primary transcript, processing of the primary and precursor microRNAs, and/or microRNA-mRNA interactions (Ryan et al., 2010). SNPs are small genetic variations in DNA sequences in which a single nucleotide is replaced by one of the other three nucleotides, for example GAATTC to GACTTC. SNPs are found mostly outside of coding regions, and occur with >1% frequency in the human population. There are ~10-30 million SNPs in the human genome, and they appear on average every 100-300 bases. The variability of SNPs between individuals makes genetic linkage analysis a powerful tool for identifying correlations between microRNAs and human disease. A free public database, dbSNP, was developed and is hosted by the National Center for Biotechnology Information in collaboration with the National Human Genome Research Institute (Sayers et al., 2010; Sherry et al., 1999; Smigielski et al., 2000; Wheeler et al., 2007). Sequence data deposited into dbSNP is assigned a unique SNP number ID (ss#), and then identical ss# records are compiled by dbSNP into one reference SNP cluster (rs#). Users can retrieve data for specific rs# records and analyze the association of genetic variation with phenotypic traits. MicroRNA SNP linkage analysis for several human diseases was enabled by multiple independent genome-wide association studies, including 194,258 disease cases and 256,754 controls (Glinsky 2008). Patterns of associations between disease-linked SNPs, microRNAs and protein-coding mRNAs revealed a human disease phenotype consisting of 81 SNPs and 17 microRNAs. A consensus set of microRNAs was associated with 4-7 human diseases. Most of the microRNAs (15/17) were predicted to target proteins in the nuclear import pathway and/or the inflammasome pathway. Indeed, expression profiling of peripheral blood mononuclear cells showed significant changes in importin alpha 5, and in the NLR family pyrin domain containing proteins 1 and 3, in phenotypes associated with Crohn’s disease, Huntington’s disease and rheumatoid arthritis (RA). Interestingly, chloroquine is a widely used for treatment of RA, malaria and systemic lupus erythematosus, and RA patients treated with chloroquine had a reversal of these phenotypes (Glinsky 2008).

Protein coding gene mutations for common psychiatric disorders have not been established. Thus, microRNA gene studies are of particular interest in psychiatric genetics. Analysis of microRNAs in schizophrenia and autism patients revealed at least 24 human X-linked microRNA variants with naturally occurring SNPs that impaired or enhanced microRNA processing, and altered processing sites (Sun et al., 2009). Genetic studies in schizophrenia patients also identified aberrant SNPs in microRNA genes as contributing factors to this disease (Cacabelos et al., 2010). A recent, in-depth review discusses the involvement of microRNAs in the pathogenesis of several psychiatric disorders (Forero et al., 2010).

Functional polymorphisms in the 3′UTRs of several genes are reported to be associated with diseases. MiRSNPs are a novel class of functional polymorphisms defined as those present at or near microRNA binding sites which interfere with microRNA function and affect gene expression. A functional miRSNP, 829C-T in the 3′UTR of dihydrofolate reductase interferes with miR-24 function which leads to over-expression of dihydrofolate reductase and methotrexate resistance (Mishra et al., 2008). Polymorphisms that affect microRNA-mediated function are also present in genes involved in microRNA biogenesis, and for processing primary microRNAs into mature microRNAs. Processing polymorphisms would likely affect expression of several genes and have more serious consequences. In contrast, polymorphisms in the microRNA or the mRNA 3′UTR would likely just affect target expression (Mishra et al., 2009).

Polymorphisms were evaluated for their association with clinical outcome in metastatic colorectal cancer patients treated with the chemotherapeutic agents, 5-fluorouracil and irinotecan (Boni et al., 2010). Eighteen SNPs analyzed in 61 patients revealed a significant association with tumor response and time to progression for SNP rs7372209 in primary miR26a-1; the genotypes CC and CT were favorable when compared with the TT variant genotype. SNP rs1834306, which is located in the primary miR-100 gene, significantly correlated with a longer time to progression. In the microRNA biogenesis pathway, a trend was also identified between SNP rs11077 in the exportin-5 gene and disease control rate. These studies suggest that the relationship between treatment outcome and SNPs in the microRNA-biogenesis machinery, for both primary and precursor microRNAs, might be useful predictors of clinical outcome in metastatic colorectal cancer patients treated with 5-fluorouracil and CPT-11 (Boni et al., 2010).

Pharmacogenetic studies show that microRNA and their predicted effectors are targets for the action of psychotherapeutic drugs For example, the mood stabilizers lithium and valproate alter the expression of hippocampal microRNAs predicted to target proteins involved in neurite outgrowth, neurogenesis, and signaling of PTEN (phosphatase and tensin homolog), ERKs (extracellular signal regulated kinases), and Wnt/beta-catenin pathways, several of which are genetic risk candidates for bipolar disorder (Zhou et al., 2009). Lithium and valproate also increased expression of potential susceptibility genes *in vivo*, including dipeptidyl-peptidase 10, metabotropic glutamate receptor 7 (mGluR7), and the thyroid hormone receptor beta. Addition of lithium or valproate to primary cell cultures decreased miR-34a expression and increased mGluR7 expression. mGluR7 is a predicted target of miR-34a, and miR-34a expression inversely correlated with mGluR7 expression (Zhou et al., 2009). There is also evidence for the regulation of human drug-metabolizing cytochromes P450 by epigenetics and microRNAs as underlying factors in variable drug responses (Gomez et al., 2009). *In silico* evaluation of microRNA binding sites in genes that affect drug response revealed miR-133 and miR-137 as potential regulators of vitamin K epoxide reductase complex subunit 1 expression, and miR-22 as a potential regulator of methylene tetrahydrofolate reductase expression (Shomron, 2010). With the emergence of databases for cell-based pharmacogenomics, such as PACdb (Gamazon et al., 2010), studies

on the effects of drugs on microRNAs and their predicted effectors will likely play a key role in the future of individualized medicine.

7. Evolution of microRNAs as diagnostic markers of disease

Our knowledge of the biological functions of human microRNAs and their clinical potential has increased immensely (Galasso et al., 2010). Accordingly, the tools for non-invasive diagnostics have expanded to include monitoring body fluid concentrations of microRNAs. MicroRNAs in serum and plasma are stable and resistant to nuclease digestion, and their expression levels in blood are reproducible and indicative of the disease state (Cortez et al., 2009; Kosaka et al., 2010; Mitchell et al., 2008). Placental microRNAs have also been discovered in maternal plasma, revealing new possibilities for non-invasive prenatal diagnosis (Hung et al., 2009). MicroRNAs are also found in microvesicles, which are plasma membrane fragments shed from virtually all cells (Deregibus et al., 2010). Microvesicles circulate in peripheral blood where they transport mRNA and proteins between cells and play a pivotal role in cell-to-cell communication. As such, they are also implicated in the processes underlying cancer, including immune suppression, metastasis, tumor-stroma interactions and angiogenesis. Mature microRNAs are differentially expressed in microvesicles and mononuclear cells isolated from the plasma of healthy individuals, and most microvesicle-specific microRNAs are predicted to target cellular differentiation of blood cells and metabolic pathways (Hunter et al., 2008).

MicroRNAs also serve as diagnostic markers in plasma and serum isolated from human patients with distinct forms of cancer (Kosaka et al., 2010). Serum levels of miR-141 can distinguish prostate cancer patients from healthy controls (Mitchell et al., 2008). The expression of muscle-specific microRNAs (miR-1, -133a, -133b and especially miR-206) is significantly higher in rhabdomyosarcoma (RMS) tumor specimens, and in RMS cell lines compared to other tumor cell lines. These studies also showed that normalized serum miR-206 expression can differentiate between RMS and non-RMS tumors (Miyachi et al., 2010).

Pancreatic adenocarcinomas exhibit extremely hypoxic signatures, and miR-210 expression is induced by hypoxia, consistent with the finding that circulating miR-210 levels are elevated in pancreatic cancer patients (Ho et al., 2010). Likewise, miR-500 is abundantly expressed in several human liver cancer cell lines and 45% of human hepatocellular carcinoma (HCC) tissues, consistent with increased miR-500 levels in serum isolated from HCC patients, and normal miR-500 levels in sera from patients after surgical treatment for HCC (Yamamoto et al., 2009). Serum microRNAs were also evaluated as biomarkers for liver pathologies revealing five microRNAs (miR-885-5p, 574-3p, -224, -215 and miR-146a) that were up-regulated in HCC and liver cirrhosis serum pools. Levels of miR-885-5p were significantly higher in patients with HCC, liver cirrhosis, and chronic hepatitis B than in healthy controls or gastric cancer patients (Gui et al., 2010). Further, detection of miR-106a and miR-17 in peripheral blood suggests that these microRNAs may serve as diagnostic biomarkers for circulating tumor cells in patients with gastric cancers (Zhou et al., 2010). MicroRNA expression has also been shown to serve as a predictor of overall survival in non-small cell lung cancer (Hu et al., 2010). Eleven serum microRNAs were altered between longer-survival and shorter-survival groups; expression of four microRNAs (miR-486, -30d, -1 and -499) was significantly associated with overall survival (Hu et al., 2010). MicroRNA expression in sputum from patients with non-small cell lung cancer revealed that miR-21 is significantly higher in cancer patients, relative to cancer-free individuals (Xie et al., 2009). Detection of mir-21 expression produced 69.66% sensitivity and 100.00% specificity in the diagnosis of lung cancer, compared with 47.82% sensitivity

and 100.00% specificity by sputum cytology, suggesting that sputum could serve as a non-invasive diagnostic for lung cancer (Xie et al., 2009).

MicroRNA expression levels also correlate with heart disease and inflammation. The most abundant microRNA in the heart, miR-1, is significantly increased in a rat model of acute myocardial infarction induced by coronary ligation, and in serum isolated from patients with acute myocardial infarction (Cheng et al., 2010). The contribution of microRNAs to innate immunity initiated by pathogens was examined using an *in vivo* model of acute lipopolysaccharide (LPS)-induced inflammation (Schmidt et al., 2009). Of the microRNAs expressed in peripheral blood leukocytes, five consistently responded to LPS-infusion. Four microRNAs were down-regulated (miR-146b, miR-150, miR-342, and let-7g) and one was up-regulated (miR-143). Target prediction revealed that transcription of interleukin-1 receptor-associated kinase 2 might be facilitated by the decreased microRNAs, while increased miR-143 might be associated with the pronounced down-regulation of the B-cell CLL/lymphoma 2 (BCL2) gene expression during LPS endotoxemia (Schmidt et al., 2009).

Although fewer in number, studies correlating microRNA expression levels in circulating fluids with brain disease and injury hold promise for these effectors as non-invasive biomarkers in neurological disorders. Recent studies examined a role for microRNAs in T cell activation in multiple sclerosis (MS), an inflammatory disease caused by demyelination of nerve axons which results in impaired ability of brain nerve cells and the spinal cord to communicate with each other. Both miR-17 and -20a were shown to modulate T cell activation genes in a knock-in and knock-down T cell model, and the same T cell activation genes were up-regulated in MS whole blood mRNA (Xie et al., 2009). MicroRNA expression in peripheral blood samples isolated from 59 treatment naïve MS patients (18 had a primary progressive, 17 a secondary progressive and 24 a relapsing remitting disease course) and 37 controls confirmed that miR-17 and -20a are significantly under-expressed in all MS subtypes (Cox et al., 2010).

MicroRNA expression in rodent brain is altered in response to *in vivo* traumatic brain injury (TBI) (Redell et al., 2009), and is consistent with evidence for altered expression of circulating microRNAs in human TBI (Redell et al., 2010). Microarray analysis revealed 108 microRNAs in human plasma isolated from healthy volunteers. Of these, 33 microRNAs decreased and 19 microRNAs increased in severe TBI patients, and 8 microRNAs were detected only in TBI plasma (Redell et al., 2010). Changes in the expression of miR-16, -92a and -765 served as markers of severe TBI within the first 24 hours after injury, and combined analysis of all three microRNAs markedly increased diagnostic accuracy of severe TBI to 100% specificity and 100% sensitivity, relative to healthy volunteers. MicroRNA expression altered in rodent brain in response to ischemic injury correlate with changes in blood microRNA expression (Jeyaseelan et al., 2008; Liu et al., 2010). Further studies revealed that microRNAs expressed in human peripheral blood served as biomarkers in the diagnosis and prognosis of stroke, and that altered microRNA levels were detectable several months after the onset of stroke (Tan et al., 2009).

MicroRNAs are differentially expressed in the hippocampus, medial frontal gyrus and cerebellum of Alzheimer's disease (AD) patient tissue (Cogswell et al., 2008). In addition, studies show differential expression of microRNAs in CSF between AD and non-affected patients (Cogswell et al., 2008). However, there was no obvious relationship between the microRNAs altered in CSF and the absolute levels in sites of AD-mediated destruction. Cogswell and colleagues propose that the microRNAs detected in CSF samples are derived from immune cells in the CSF, consistent with predictions that microRNAs decreased in AD CSF target proteins involved in T lymphocyte signaling and inflammatory pathways (Cogswell et al., 2008). MicroRNAs are also potential biomarkers of manic episodes in

bipolar disorder (BD). The brain specific miR-134 was examined in BD patients because it was recently identified as a potential regulator of dendritic spine volume and synapse formation (Schratt et al., 2006). Plasma levels of miR-134 were significantly decreased in drug-free, 2-week and 4-week medicated bipolar mania patients, relative to control patients. Treatment with mood stabilizers increased the level of miR-134. Thus, decreased plasma miR-134 correlates with the pathophysiology and severity of manic symptoms in BD, suggesting that plasma miR-134 could serve as a non-invasive biomarker in acute manic episodes (Rong et al., 2010).

8. Current challenges to identify and validate microRNA targets in mammalian systems

This review would be remiss to leave the reader with the impression that altered expression of microRNAs is sufficient to show correlation with a specific disease. Several studies have used microarrays coupled with qRT-PCR to show altered expression of microRNAs in neurological disorders, followed by computational methods to identify predicted mRNA targets of the altered microRNAs (Saugstad, 2010). But the imperfect complementarity of animal microRNAs with their targets has made it difficult to accurately predict true targets. Moreover, proving a causal relationship between a microRNA, a predicted mRNA target, and a disease is a formidable challenge. A useful conceptual guide for establishing such a microRNA-target-disease link was provided in 1890 by Robert Koch, who proposed four criteria designed to establish a causal relationship between a microbe and a disease (Koch, 1890). Similar to Koch's postulates, Kuhn and colleagues (2008) proposed four criteria to establish a causal relationship between a microRNA, an mRNA targets, and a biological function (Kuhn et al., 2008a). The first criterion is to experimentally validate the putative interaction between microRNAs and predicted mRNA targets. This is typically done using reporter assays wherein candidate microRNAs and a vector containing a reporter (e.g., luciferase) cloned proximal to a target mRNA 3′UTR are co-transfected into cells, and the effect of the microRNAs on expression of the reporter is examined. Studies to establish a true mRNA target site involves mutagenesis of the predicted target site(s) and loss of translational repression by the microRNAs. One challenge of reporter assays is that microRNA-regulated mRNAs show a tendency to contain 3′UTR sequences longer than non microRNA-regulated mRNAs (Majoros et al., 2007), and thus cloning the entire 3′UTR into a reporter vector or transfecting the vectors into cells may be more difficult. However, one resource for transfection-ready reporter assay reagents such as genome-wide collections of promoter and 3′UTR reporter constructs is SwitchGear Genomics (http://switchgeargenomics.com/), and commercial access to these important tools will undoubtedly become more widely available in the near future.

The second criterion is to experimentally validate that microRNAs and mRNA targets are co-expressed in the same cells. Methods such as qRT-PCR and RNA blot analysis performed in a homozygous cell population are frequently used to examine temporal- and tissue-specific expression of RNA. *In situ* hybridization can establish the expression of RNAs in distinct cell types within a tissue, and theoretically, the use of RNA probes coupled to fluorescent tags could allow co-labeling of the microRNA and predicted mRNA target in the same tissue. But the distinct properties of microRNAs and mRNAs need to be considered in order to obtain accurate and meaningful data. For example, recent studies from the Tuschl lab showed that a significant portion of tissue-bound microRNAs are washed off in the hybridization buffer when using conventional *in situ* methods (Pena et al., 2009). The authors also report a modified *in situ* hybridization method that significantly increased retention of the microRNAs in tissue. Such a rigorous and detailed approach is essential for valid studies on microRNAs.

The third criterion is to experimentally validate that a given microRNA has a predictable effect on target protein expression. These experiments typically employ methods in which microRNAs are overexpressed or knocked down, and the effect on target protein expression is examined by immunoblot, immunocytochemistry, or ELISA assays, as well as binding or enzymatic studies. Recent proteomic studies combined mass spectrometry and stable isotope labeling of cells, SILAC, to examine the effects of the miR-17-92 microRNA cluster, which is often activated in cancer cells, on multiple key effectors in the Transforming growth factor-beta signaling cascade (Mestdagh et al., 2010). The fourth criterion is to experimentally validate that microRNA-mediated regulation of target gene expression equates to altered biological function. Depending on the target, such assays could include the effect of microRNAs on altered signaling pathways, cell proliferation, cell differentiation, cell death, or cell migration.

While it would be desirable to establish all four criteria for a microRNA and its predicted mRNA target, it is likely not realistic given the challenges of each criterion. For example, studies initiated on miR-125b were based on prior studies which showed that overexpression of miR-125b was the only consistent abnormality in human patients with acute myeloid leukemia (Bousquet et al., 2008) or B-cell acute lymphoid leukemia (Chapiro et al., 2010; Sonoki et al., 2005). Also, miR-125b-2 was overexpressed in patients with Down Syndrome (Trisomy 21)-associated with megakaryoblastic leukemia (Klusmann et al., 2010). These studies strongly suggested a role for miR-125b in several types of leukemia, and Bousquet and colleagues showed that indeed, overexpression of miR-125b in a mouse model also induced leukemia (Bousquet et al., 2010). The target(s) of miR-125b remain to be validated. Such validation data might potentially be mined from the literature. A new database, known as miRTarBase, reports microRNA-target interactions that have been experimentally validated by the methods discussed herein (Hsu et al., 2010). Information in miRTarBase results from manually surveying pertinent literature after systematic data mining of the text to filter research articles related to functional studies of microRNAs. To date, miRTarBase (http://miRTarBase.mbc.nctu.edu.tw/, accessed Jan 1, 2011) has accumulated more than 3,500 microRNA-target interactions between 657 microRNAs and 2297 mRNA targets among 17 species, and the database is frequently updated by continuously surveying research articles. Development of such valuable resources will certainly expedite studies to validate interactions between microRNAs and their targets, to correlate these data with biological functions, and to potentially translate this information into novel therapies for the treatment of diseases.

9. Conclusions

MicroRNAs regulate the expression of more than half of all genes, and they are essential for nervous system development and function. Thus, correlations between dysregulation of microRNAs and brain diseases are to be expected. Most examples of microRNAs as circulating non-invasive biomarkers for disease are peripheral to the brain, yet there is accumulating evidence for altered microRNA expression in the blood and CSF in brain injury, neurodegeneration and psychiatric disorders. These studies support that microRNAs offer a promising means for non-invasive monitoring of disease progression in brain disorders. The continued rapid evolution of prediction tools, criteria for experimental validation, and databases for public access of this information are essential to establish a correlation between microRNAs and disease.

Acknowledgments

We thank Dr. Michael Roberts for his comments and superb editing which greatly improved this manuscript, the NIH for generous support by grants R21NS054220, R01NS064270 (JAS) and the Spastic Paralysis Research Foundation of the Illinois-Eastern Iowa District of Kiwanis International for a grant [SC].

Abbreviations

APT1	Acyl protein thioesterase 1
AD	Alzheimer's disease
Ago	Argonaute
ASD	Autism spectrum disorder
BD	Bipolar disorder
CSF	Cerebrospinal fluid
eIF	Eukaryotic initiation factor
FMRP	Fragile X mental retardation protein
FXR1P/FXR2P	Fragile X-related proteins 1 or 2
Hsa21	Human chromosome 21
HCC	Human hepatocellular carcinoma
LIMK1	LIM Kinase 1
LPS	Lipopolysaccharide
LTP	Long term potentiation
LTD	Long term depression
mRNA	Messenger RNA
mGluR	Metabotropic glutamate receptor
MS	Multiple sclerosis
PUM	Pumilio proteins
PUF	Pumilio-Fem3 binding factor
qRT-PCR	Quantitative real-time PCR
RMS	Rhabdomyosarcoma
RNase	Ribonuclease
RISC	RNA-induced silencing complex
SNP	Single nucleotide polymorphism
TBI	Traumatic brain injury
5′UTR/3′UTR	5′ or 3′ untranslated region

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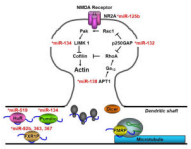

Figure 1. MicroRNA Regulation of Key Proteins at the Dendrite

From left: miR-134 suppresses LIMK1 translation at the synapse, while miR-132 suppresses p250GAP, an inhibitor of the Rho family GTPases. Acyl protein thioesterase 1 (APT1), an enzyme that regulates the palmitoylation status of synaptic proteins, is regulated by miR-138. In the dendritic shaft: RNA-binding proteins that regulate transport, stability and/or translation of mRNAs. Pumilio2 is regulated by miR-134, HuR is regulated by miR-519 and autosomal paralog of FMRP, FXR1P, is regulated by miRNAs 92b, 363 and 367. FMRP is present in microtubule-associated granules at the dendrite, and is shown associated with mRNAs and in a complex with microRNAs and precursor microRNAs. Dicer has also been reported in dendrites. Top of the spine, miR-125b regulates expression of the NR2A receptor, a component of the NMDA receptor.