Published in final edited form as:

Neurotoxicology. 2012 June ; 33(3): 530–544. doi:10.1016/j.neuro.2012.02.013.

Non-coding RNAs—Novel targets in neurotoxicity

Tamara L. Tal* and Robert L. Tanguay

Department of Environmental and Molecular Toxicology and the Environmental Health Sciences Center, Oregon State University, Corvallis, OR, United States

Abstract

Over the past ten years non-coding RNAs (ncRNAs) have emerged as pivotal players in fundamental physiological and cellular processes and have been increasingly implicated in cancer, immune disorders, and cardiovascular, neurodegenerative, and metabolic diseases. MicroRNAs (miRNAs) represent a class of ncRNA molecules that function as negative regulators of post-transcriptional gene expression. miRNAs are predicted to regulate 60% of all human protein-coding genes and as such, play key roles in cellular and developmental processes, human health, and disease. Relative to counterparts that lack binding sites for miRNAs, genes encoding proteins that are post-transcriptionally regulated by miRNAs are twice as likely to be sensitive to environmental chemical exposure. Not surprisingly, miRNAs have been recognized as targets or effectors of nervous system, developmental, hepatic, and carcinogenic toxicants, and have been identified as putative regulators of phase I xenobiotic-metabolizing enzymes. In this review, we give an overview of the types of ncRNAs and highlight their roles in neurodevelopment, neurological disease, activity-dependent signaling, and drug metabolism. We then delve into specific examples that illustrate their importance as mediators, effectors, or adaptive agents of neurotoxicants or neuroactive pharmaceutical compounds. Finally, we identify a number of outstanding questions regarding ncRNAs and neurotoxicity.

Keywords

microRNAs; ncRNAs; Neurotoxicology

1. Non-coding RNAs

In contrast to protein-coding genes, the number of encoded non-coding RNAs (ncRNAs) increases proportionally with increasing developmental complexity (Taft et al., 2007). Thus, ncRNAs have been shown to enhance the phenotypic complexity of eukaryotic organisms that often share similar numbers of protein coding genes (Mattick, 2004). With the exception of ncRNAs that play specific roles in the synthesis, splicing, and transport of proteins like ribosomal RNA and transfer RNA, ncRNAs were, until recently, thought to represent transcriptional noise. Interestingly, genes that contain large amounts of intronic sequences are often highly expressed in the nervous system or downregulated in embryonic stem cells and cancers, suggesting that increasing organismal complexity may be explained by the expansion of cis-acting regulatory elements and both cis- and trans-acting ncRNAs (Taft et al., 2007).

© 2012 Elsevier Inc. All rights reserved

*Corresponding author. Present address: Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Research Triangle Park, NC 27711, United States. Tel.: +1 919 541 0506; fax: +1 919 541 0717. tal.tamara@epa.gov (T.L. Tal)..

Conflict of interest The authors declare that they have no competing financial interest.

Although the field of ncRNAs and neurotoxicology is still in its infancy, particularly with regard to environmental agents, the goal of this review is to summarize the current state of the field. We additionally drew on advancements made in the neuro-active pharmaceuticals and drugs of abuse literature in order to describe the myriad of mechanisms through which exogenous compounds exert neurotoxicity via disruptions in ncRNA homeostasis. This review primarily focuses on a specific class of ncRNAs called microRNAs (miRNAs) and the multiplicity of roles that these negative regulators of gene expression play as agents of neurotoxicants or neuroactive pharmaceuticals. Though not nearly as well understood, mechanisms by which other classes of ncRNAs influence neurotoxicity will also be briefly discussed.

2. The basics of miRNA transcription, biogenesis, stability and function

The most well understood ncRNA system is post-transcriptional regulation of gene expression by miRNAs. Though the term “microRNA” was coined after their initial discovery in plants (Hamilton and Baulcombe, 1999), miRNAs are historically most well known for their role as regulators of developmental timing in the nematode *Caenorhabditis elegans* (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). In mammals, miRNAs are predicted to exert post-transcriptional control over 60% of protein coding genes (Friedman et al., 2009). It is therefore not surprising that they have been associated with a broad spectrum of cellular and developmental processes including developmental timing, hematopoiesis, organogenesis, apoptosis, and cell proliferation (Kim, 2005). miRNAs are short (~22 nucleotides), single stranded RNAs that control post-transcriptional gene expression by binding to complementary sequences in the 3' untranslated region (3'-UTR) of target mRNA transcripts, termed miRNA recognition elements (MREs). Binding generally results in message repression by facilitating translational repression or deadenylation and degradation (Kim et al., 2009). Interestingly, it has recently been proposed that message containing 3'-UTR motifs targeted by specific RNA binding proteins are more likely to be up- or down-regulated following cellular transfection with miRNAs (Jacobsen et al., 2010). Genes upregulated after miRNA transfections tend to contain an ARE “stability motif” and attenuate miRNA-mediated repression by transfected miRNAs while downregulated transcripts are more likely to contain one of two novel U-rich motifs that function to augment repression by transfected exogenous miRNAs (Jacobsen et al., 2010). miRNAs typically bind to target MREs with imperfect complementarity, yet with perfect complementarity in the seed region comprised of nucleotides (nt) 2–7 from the 3' end of the molecule (Brennecke et al., 2005). Much less frequently, transcripts whose MREs bind miRNAs with near perfect complementarity undergo endonucleolytic cleavage and degradation. While rare in animals, this is the predominant mode of miRNA action in plants (Brennecke et al., 2005).

miRNA biogenesis has been the subject of a number of excellent reviews (Du and Zamore, 2005; Kim et al., 2009; Krol et al., 2010b; Kim, 2005), and will therefore only be briefly discussed here (Fig. 1). miRNAs are transcribed by RNA polymerase II. Depending on location, primary miRNA (pri-miRNA) may be transcribed independently or, if they reside in introns or exons, along with their host genes. In the case of autonomously expressed miRNAs, transcription is mediated by promoter regions that contain CpG islands, TATA box sequences, initiation elements and histone modifications. This indicates that, similar to protein-coding genes, miRNA promoters are controlled by transcription factors, enhancers, silencing elements and chromatin modifications (Corcoran et al., 2009). A host of transcription factors positively or negatively regulate miRNA transcription in a tissue- or developmental stage-specific manner, and transcribed miRNAs can, in turn, post-transcriptionally regulate transcription factor levels via negative feedback loops (Fig. 6, reviewed in Krol et al., 2010b).

In the canonical biogenesis pathway, the nuclear RNase III enzyme Drosha in complex with DGCR8, a ds-RNA binding protein, produces ~70 nt pre-miRNA (Lee et al., 2003). Following nuclear export via exportin 5, pre-miRNA molecules are bound in the cytoplasm by a second RNase III enzyme termed Dicer in complex with the transactivation-response RNA-binding protein (TRBP). Approximately 50% of miRNAs are located in short introns (mitrons, Fig. 1) of protein-coding genes (Hinske et al., 2010), and a small number of miRNAs map to exons. Following splicing and debranching, mitrons give rise to precursor miRNA (pre-miRNA) hairpin structures that bypass the first step in canonical miRNA biogenesis, RNase III Drosha-mediated cleavage. The biogenesis pathway ultimately gives rise to mature miRNAs that associate with a number of miRNA-binding proteins in the miRISC complex to post-transcriptionally repress target mRNAs (Fig. 1).

A key component of the miRISC complex are the Argonaute proteins which bind the 3' and 5' ends of the miRNA via evolutionarily conserved PAZ, MID, and PIWI domains (Fig. 1 and Fabian et al., 2010). miRNA are presented on the AGO protein surface to engender base pairing with target mRNA (Fabian et al., 2010). GW182 proteins are another miRISC component and function in part to directly bind AGO-bound miRNA and mRNA and contain a RNA recognition motif that mediates translational repression and deadenylation of mRNA (Fig. 1 and Fabian et al., 2010). GW182 proteins also contain a domain that traffics post-transcriptionally repressed mRNAs to P-bodies, discrete cytoplasmic foci that are enriched in proteins involved in mRNA deadenylation, decapping, and degradation (Parker and Sheth, 2007). Several other molecules beyond the core components of miRISC (AGO and GW182) are involved in multiple aspects of miRNA function (summarized in Krol et al., 2010b).

miRNA levels are additionally controlled by miRNA turnover. In *C. elegans*, the 5'–3' exonuclease XRN-2 catalyses the degradation of mature miRNAs (Chatterjee and Grosshans, 2009). Although the turnover process is not fully understood, mature miRNA must first be released from the miRISC complex in order to allow XRN-2 enzymatic access to the miRNA (Chatterjee and Grosshans, 2009). Liberation of mature miRNAs from miRISC is blocked if the miRNA is additionally bound to its mRNA target (Chatterjee and Grosshans, 2009). As may be expected from the mRNA literature, the half-lives of miRNA vary greatly depending on elements within the miRNA sequence, cell type, cell cycle, or by multiple modifications of the 3' end that stabilize or destabilize the molecule (Krol et al., 2010b; Grosshans and Chatterjee, 2010). Some of the shortest miRNA half-lives reported to date have been observed in neurons (Krol et al., 2010b) where rapid miRNA turnover has been noted in response to dark adaptation and activity-dependent signaling (Krol et al., 2010a). The effects of chemical exposures on miRNA transcription, decay, or destabilization have not been well studied, although there is some suggestion that neuroactive compounds may elicit their activities through diverse miRNA regulation mechanisms (Kocerha et al., 2009).

3. Other ncRNAs

Beyond miRNAs, there are numerous other classes of ncRNA that are arbitrarily classified as either short or long ncRNA based on an approximate cutoff of 200 bases (Table 1). These short- and long-ncRNAs are produced endogenously, via transcription or splicing mechanisms, or introduced from exogenous sources. In animals, short ncRNAs include small nucleolar RNAs (snoRNAs), small vault RNAs (svRNAs), and a number of promoter-associated small RNAs listed in Table 1. These classes of small ncRNAs are generally processed by dicer, a key ribonuclease involved in miRNA biogenesis (Fig. 1), but bypass cleavage by Drosha/DGCR8 and play diverse roles in post-transcriptional regulation of transcripts and transposons and anti-viral defense (reviewed in Ghildiyal and Zamore, 2009).

One exception to this rule is the class of short piwi-interacting RNAs (piRNAs) that are produced independently of dicer and bind to the PIWI subfamily of AGO proteins to maintain genome stability in germline cells by silencing transposons (Aravin et al., 2007). In comparison to short ncRNAs, long ncRNAs (lncRNAs) remain largely uncharacterized. lncRNAs are transcripts generally > 200 nucleotides that are known to influence chromatin modifications involved in X-chromosome inactivation, and function as direct and indirect regulators of transcriptional and translational regulation, as molecular chaperones that enable protein conformational activity, and as transporters that allow protein transport and localization (Table 1, reviewed in Au et al., 2011).

4. ncRNAs regulate neurodevelopment and disease

It has been proposed that gene regulatory networks in the brain are comprised of feed-forward and feedback loops that are sensitive to amplification or restriction by miRNAs (Tsang et al., 2007). Perhaps among the most well studied aspect of the brain with regard to miRNAs is its development. The developing nervous system is a rich source of miRNAs (Kapsimali et al., 2007). Indeed, miRNA expression profiling studies have identified a number of miRNAs that are enriched in the nervous system and whose expression is tightly controlled, both spatially and temporally, during development (Kapsimali et al., 2007; Krichevsky et al., 2003). In animal studies, genomic ablation of dicer triggers loss of dicer-dependent miRNA synthesis resulting in severe deficits in brain development (De Pietri Tonelli et al., 2008; Giraldez et al., 2005; Huang et al., 2010). In zebrafish, maternal-zygotic dicer mutants display defects in central nervous system (CNS) patterning that are partially rescued by exogenous miR-430, underscoring the significance of miRNAs in brain morphogenesis (Giraldez et al., 2005). Dicer ablation has also been correlated with neurodegeneration (Schaefer et al., 2007), neuronal cell death (De Pietri Tonelli et al., 2008), survival of differentiating neural crest cells (Zehir et al., 2010), and synaptic plasticity including deficits in dendritic arborization (Davis et al., 2008) and axon pathfinding (Pinter and Hindges, 2010). Interestingly, mice lacking dicer in specific dopamine neurons survive but experience progressive loss of dopaminergic neurons later in life and consequently develop what has been termed a “Parkinson's-like” phenotype (Hebert et al., 2010).

miRNAs are now known to play critical roles in the pathogenesis of neurodegeneration and disease. Differential expression of miRNAs has been reported in Alzheimer's disease, Parkinson's disease, ataxia, Huntington's disease and schizophrenia patients and/or models (Lau and de Strooper, 2010). As demonstrated for neurodevelopment, there is evidence that defects in miRNA biogenesis are associated with multiple different neurological disorders. Indeed, mutations in varied components of the miRNA biogenesis pathways are correlated with aspects of amyotrophic lateral sclerosis (Ling et al., 2010), Fragile X Syndrome (Li and Jin, 2009), and Parkinson's disease (Gehrke et al., 2010). In humans, individuals with 22q11.2 microdeletions are at high risk of developing schizophrenia that may result from haploinsufficiency of the DGCR8 gene and subsequent deficits in Drosha/DGCR8-mediated miRNA biogenesis (Stark et al., 2008). A number of specific miRNAs have also been linked to these same neurological diseases (reviewed in Esteller, 2011). In addition, lncRNAs are increasingly associated with neurodegeneration (Derrien et al., 2011). For example, the lncRNA BACE-AS, involved in the pathophysiology of Alzheimer's disease, binds and stabilizes BACE-1 mRNA resulting in elevated protein levels and subsequent rise in the production of B-amyloid peptides (Faghihi et al., 2008). However, with few exceptions, little is known regarding the possible role that ncRNAs may play in toxicant-mediated neurological disease.

5. miRNAs and dendritic plasticity

Synaptic plasticity is critical to neurodevelopment, learning, and memory. Dendritic spines are small, post-synaptic protrusions from neuronal dendrites that serve as the primary recipients of excitatory input in the CNS (reviewed in Bourne and Harris, 2008). Neuronal activity regulates the number, morphology, and composition of dendritic spines, together comprising the structural basis for synaptic plasticity during development, learning, and memory (Bourne and Harris, 2008). In addition to changes in gene expression, localized regulation of protein synthesis is required for synaptic plasticity (reviewed in Klann and Dever, 2004). miRNAs participate in localized modifications of protein translation that regulate structural changes underlying plasticity. Indeed, mRNA transcripts, pre-miRNAs, dicer, and miRISC components are trafficked to dendritic spines to facilitate controlled formation of mature miRNAs that exert localized translational regulation (Lugli et al., 2005). In dendrites, miRNA-repressed mRNA, AGO proteins, and translational repressors further localize to dendritic P-body-like particles (Barbee et al., 2006; Cougot et al., 2008). Interestingly, dendritic synthesis of mature miRNAs appears to rely on activity-dependent stimulation of the protease calpain that in turn cleaves dicer to unleash its formerly constrained RNase III activity (Lugli et al., 2005; Smalheiser and Lugli, 2009).

Perhaps the most well studied dendritically enriched miRNA is miR-132 which promotes dendritic spine morphogenesis and branching in cortical (Vo et al., 2005) and hippocampal (Impey et al., 2010; Wayman et al., 2008) neurons. In hippocampal neurons, miR-132 functions as an activity-dependent rapid response gene regulated by the CREB signaling pathway (Wayman et al., 2008). miR-132 represses translation of the Rho family GTPase-activating protein, p250GAP (Wayman et al., 2008), thereby releasing its inhibition of Rac1 (Impey et al., 2010). This allows Kalirin-7-mediated activation of Rac1 and subsequent activation of Pak driven actin polymerization and spine formation (Impey et al., 2010). In support of these studies, deletion of miR-132 blocks dendritic growth and branching of immature neurons in the adult hippocampus (Magill et al., 2010), while miR-132 overexpression increases dendritic spine density in the forebrain, reduces methyl CpG binding protein 2 (MeCP2) levels, and is associated with deficits in novel object recognition (Hansen et al., 2010). Taken together, these studies describe an intricate activity-dependent circuit through which miR-132 promotes dendritic morphogenesis.

In contrast to miR-132, a number of miRNAs negatively regulate dendritic morphogenesis and branching. In one of the earliest reports describing the regulation of dendritic spine development by a dendritically enriched miRNA, Schratt et al. showed that miR-134 negatively regulates the size of post-synaptic dendritic spines in hippocampal neurons (Schratt et al., 2006). Its inhibitory actions are mediated by direct post-translational repression of *limk1* mRNA levels, a kinase that is upregulated by BDNF-dependent signaling and functions to promote dendritic spine development (Schratt et al., 2006). Though it does not impact dendritic density, miR-138 also negatively influences spine size (Siegel et al., 2009). Siegel and colleagues report that, in the absence of miR-138, acyl protein thioesterase 1 (APT1) is expressed in dendritic spines and depalmitoylates synaptic proteins including Gα13, preventing membrane localization (Siegel et al., 2009). miR-138 represses APT1 translation, allowing for unrestricted Gα13 palmitoylation and membrane localization, ultimately resulting in spine shrinkage. In contrast, the activity-dependent miRNAs miR-29a and miR-29b impair dendritic spine head enlargement and synaptic consolidation via post-transcriptional regulation of a subunit of the ARP2/3 actin nucleation complex that is involved in dendritic spine morphogenesis (Lippi et al., 2011). In summary, a number of miRNAs control structural aspects of dendritic spines and likely play a significant role in activity-mediated synaptic plasticity.

6. miRNAs, nuclear receptors, and drug metabolism

Cytochrome P450s are the major enzymes that catalyze the metabolism of xenobiotics including environmental chemicals, pharmaceutical agents, and drugs of abuse. A number of P450s are post-transcriptionally regulated by miRNAs including CYP1B1, CYP2A3, CYP2E1, CYP3A4 and CYP24A1 (reviewed in Nakajima and Yokoi, 2011; Yu, 2009). miRNAs additionally target several upstream nuclear receptors including PXR, RXR α , GR, ER α , PPAR γ , VDR and HNF α (Nakajima and Yokoi, 2011). CYP3A4, one of the most prolific drug-metabolizing P450s (Guengerich, 2008), provides an interesting example of the complexity of miRNA-mediated regulation of xenobiotic metabolism. In 293T cells, repression of a reporter gene harboring the CYP3A4 3'-UTR was blocked by mutating miR-27b or miR-298 MREs demonstrating that miR-27b and miR-298 directly repress CYP3A4 mRNA levels (Pan et al., 2009). CYP3A4 is transcriptionally regulated by PXR and VDR and these transcription factors are also under the post-transcriptional control of miRNAs (Pan et al., 2009). In addition, CYP3A4 is post-transcriptionally regulated by the small vault RNA svRNA β (Persson et al., 2009). This novel finding may shed light on the previously unexplained association between vault particles and drug resistance (Persson et al., 2009). Similar to CYP3A4, VDR transcripts are targeted by miR-27b and miR-298 and over-expression of the miRNAs reduces cellular sensitivity to the alkylating agent cyclophosphamide in PANC1 cells (Pan et al., 2009). In the case of PXR, *in vitro* studies showed that PXR is a bona fide target of miR-148a, which can attenuate PXR-dependent CYP3A4 expression (Takagi et al., 2008). In a panel of 25 human livers, miR-148a levels were inversely correlated with levels of PXR mRNA and protein (Takagi et al., 2008). These studies indicate the miRNAs influence drug metabolism by directly regulating CYP3A4 levels and indirectly by repression of VDR- or PXR-dependent CYP3A4 expression. In a broader sense, these studies illustrate the ability of different types of ncRNAs to exert effects at multiple levels within the same signaling pathway.

7. Neurotoxicology of miRNAs

When compared with transcripts that lack MREs in their 3'-UTRs, mRNAs that are post-transcriptionally regulated by miRNAs are twice as likely to be sensitive to changes in expression levels following exposure to environmental chemicals (Wu and Song, 2011). This suggests that ncRNAs, with their short half-lives (Krol et al., 2010b) and their ability to post-transcriptionally regulate hundreds of transcripts, are well placed to rapidly respond to a wide array of environmental disturbances, including chemical exposures. The following sections describe studies that expand our understanding of the roles that ncRNAs play in mounting adaptive responses to neurotoxicant exposure or as effectors of toxicity or drug efficacy (Table 2).

8. Neuroactive pharmacological agents: miRNAs regulate drug efficacy and tolerance

Pharmacological compounds that directly involve miRNAs in their modes of action or indirectly by their roles in adaptive responses to drug exposure include a broad array of neuroactive agents including antivirals, glucocorticoids, alkylating agents, antidepressants and mood stabilizers.

8.1. Antivirals

The antiviral acycloguanosine acyclovir is used to treat herpes simplex virus-1 (HSV-1), a virus that establishes lifelong latency in the human nervous system. The establishment of HSV-1 latency and suppression of host cellular immune responses involves the expression of a suite of virally encoded miRNAs (Umbach et al., 2008) in addition to virally induced

changes in host miRNA levels (Skalsky and Cullen, 2010). It has been proposed that antiviral agents exert their activities through modulation of host miRNAs (Lukiw et al., 2010). Preliminary studies in mixed cultures of primary human neuronal and glial cells show that acyclovir treatment blocks HSV-1-induced expression of miR-146a (Lukiw et al., 2010), a miRNA that is upregulated following HIV infection and involved in regulation of the innate immune response (Rom et al., 2010). More work is needed to determine whether acyclovir-induced changes in miR-146a levels impact viral pathogenesis or latency. As will be discussed later on, increased levels of miR-146a also appear to be a sensitive indicator of cellular stress following exposure to a number of neurotoxic metals (Lukiw and Pogue, 2007; Lukiw et al., 2008; Pogue et al., 2009). It will be interesting to determine whether miR-146a upregulation serves as a common cellular response to other chemical or pathogenic insults beyond metals and viral infection.

8.2. Glucocorticoids

Glucocorticoids impact a broad array of physiological processes including nervous system development, adaptation to stress, metabolism, and immunity. The glucocorticoid receptor (GR) itself is subject to post-transcriptional control by miRNAs (Vreugdenhil et al., 2009). The brain-enriched, miR-124a posttranscriptionally regulates GR mRNA levels in differentiating P19 cells (Vreugdenhil et al., 2009). In developing hippocampal neurons, the glucocorticoid dexamethasone (DEX) blocks synaptic maturation and excitatory neurotransmitter glutamate release enhanced by brain-derived neurotrophic factor (BDNF)-mediated mitogen activated protein kinase signaling (MAPK) (Kumamaru et al., 2008). Deep sequencing revealed that the miR-212/132 cluster, containing miR-132, miR-132*, miR-212 and miR-212*, is responsive to BDNF treatment in cultured primary cortical mouse neurons, and that upregulation of these miRNAs by BDNF exposure relies on ERK1/2 (extracellular signal-related kinase 1/2) signaling (Remenyi et al., 2010). In support of this, increased levels of post-synaptic proteins in BDNF-treated in hippocampal neurons are dependent on ERK1/2-mediated upregulation of miR-132 (Kawashima et al., 2010), a miRNA critical for normal dendrite maturation in newborn neurons (Magill et al., 2010). miR-132 levels in turn, are suppressed by exogenous glucocorticoid exposure, thus preventing BDNF-induced synaptic maturation (Kawashima et al., 2010). Together, these studies illustrate a feed-forward mechanism, whereby synaptic maturation is influenced by BDNF-dependent increases in miR-132 levels that can be blocked at the level of miR-132 by glucocorticoid treatment. More work is needed to determine whether other members of the miR-212/132 cluster are similarly involved in synaptogenesis, upregulated by ERK1/2-dependent signaling, and repressed by glucocorticoid exposure.

8.3. Chemotherapeutics

As an example of the contribution of miRNAs to drug resistance in the nervous system, the pro-apoptotic effects of the chemotherapeutic temozolamide (TMZ) are tempered by reprogramming miR-21 expression in human glioma cells (Shi et al., 2010). TMZ is a methylating agent that triggers apoptosis in malignant glioma cells (Roos et al., 2007). While effective, developed resistance to the drug is common. In cultured human glioblastoma cells, TMZ disrupts the balance of Bax and Bcl-2, pro-apoptotic and anti-apoptotic molecules, respectively, whose ratio controls activation of cellular apoptosis, thereby favoring the development of drug resistance (Ma et al., 2002). Using loss- and gain-of-function approaches, Shi and colleagues showed that increased levels of miR-21 confers partial resistance to the killing effects of TMZ while miR-21 knockdown increases cell susceptibility to TMZ-induced apoptosis (Fig. 2, Shi et al., 2010). miR-21 is a putative miRNA oncogene that is upregulated in human glioblastoma tumor tissues and functions as an anti-apoptotic factor (Chan et al., 2005). Mechanistically, increased levels of miR-21 trigger reductions in Bax and increased Bcl-2 levels, and these changes are accompanied by

reductions in caspase-3 levels and programmed cell death (Shi et al., 2010). These data suggest that glioblastoma cells, primed with increased miR-21 levels, have a concomitant reduction in baseline susceptibility to apoptosis thereby blunting the killing effects of TMZ. miR-21 knockdown results in the opposite effect in glioblastoma cells: reduced Bcl-2, increased caspase 3, and heightened sensitivity to the cell killing effects of taxol (Ren et al., 2010).

In a number of medulloblastoma cell lines, resistance to mitomycin C and cisplatin was inversely correlated with levels of the p53 tumor suppressor transcript and miR-34a (Weeraratne et al., 2011). miR-34a directly targets and represses the oncogenic MAGE-A transcript and allows for a concomitant elevation in p53 levels, while p53 in turn, upregulates miR-34a (Weeraratne et al., 2011) in what proves to be an elegant positive feedback circuit (Fig. 6). p53 also enhances the maturation of the growth suppressive miRNAs miR-16-1, miR-143, and miR-145 (Suzuki et al., 2009). Taken together, these studies add to a growing body of literature describing miRNA-mediated drug resistance in breast (Miller et al., 2008) and biliary tract cancer cells (Meng et al., 2006), and underscore the relevance of targeting cellular miRNAs as a strategy to increase the efficacy of preexisting chemotherapeutics.

The non-coding vault RNAs (vRNAs) are implicated in cancer cell resistance to chemotherapeutic exposure (Gopinath et al., 2005, 2010). vRNAs are components of large ribonucleoprotein vault particles that collectively participate in intracellular and nucleocytoplasmic transport as well as the extrusion of xenobiotics from nuclei of resistant cancer cells. vRNAs directly bind mitoxantrone (Gopinath et al., 2005), and human glioblastoma, leukemia, and osteocarcinoma cell lines overexpress vRNA and exhibit higher resistance toward mitoxantrone treatment (Gopinath et al., 2010). vRNAs fold into secondary structures resembling miRNA precursors and give rise to small vRNAs (svRNAs) via a mechanism independent of Drosha but dependent on Dicer activity (Persson et al., 2009). One of these, svRNAb, associates with AGO to post-transcriptionally repress levels of CYP3A4, a key enzyme in drug metabolism (Persson et al., 2009), providing an intriguing possibility underlying the long observed association between vault particles and drug resistance. Together, these studies point to a number of mechanisms by which vRNAs may confer drug resistance in cancer.

8.4. Antidepressants and mood stabilizers

Selective serotonin reuptake inhibitor (SSRI) antidepressants target transporters specific for serotonin (SERTs) to block monoamine reuptake at the synaptic cleft. Adaptive reductions in SERT protein levels following chronic SSRI antidepressant treatment is a consequence of reduced SERT at the post-transcriptional level (Benmansour et al., 2002). A study by Baudry and colleagues provides evidence supporting a mechanism through which the SSRI fluoxetine (Prozac) reduces SERT expression in serotonergic neurons by increasing the maturation of its negative regulator miR-16 from precursor (pre)-miR-16 molecules (Baudry et al., 2010). They show that miR-16 functions to repress SERT transcript levels both in serotonergic cells and *in vivo*, in serotonergic raphe nuclei (Baudry et al., 2010). In support of the concept that miRNAs mediate drug efficacy, chronic infusion of fluoxetine or miR-16 into raphe nuclei partially blocks a host of behavioral phenotypes in mice subjected to unpredictable chronic mild stress (Baudry et al., 2010). Based on pharmacological and behavioral data, the authors suggest that miR-16 positively contributes to the therapeutic actions of SSRI antidepressants in serotonergic neurons (Baudry et al., 2010).

In one of the earliest reports showing that miRNAs are the targets of psychotherapeutics, the mood stabilizers lithium and valproate were shown to elicit efficacy, in part, via miRNA-mediated repression of the metabotropic glutamate receptor 7 (GRM7) (Zhou et al., 2009).

GRM7 is a candidate gene recently identified by whole genome wide association study in bipolar patients (Alliey-Rodriguez et al., 2011). Zhou et al. showed that a number of miRNAs are misregulated in rat hippocampal tissue upon chronic exposure to lithium or valproate, and that miR-43a levels are reduced (Zhou et al., 2009). A number of putative target genes, known to be genetic risk candidates for bipolar disease, were identified that were both differentially expressed upon treatment with the psychotherapeutics and predicted to be post-transcriptionally regulated by several inversely expressed miRNAs, including GRM7 (Zhou et al., 2009). Ectopic expression of the miR-34a precursor down-regulated levels of its putative target GRM7 in primary cultures of rat hippocampal neurons, phenocopying the effect of lithium or valproate exposure on GRM7 expression (Zhou et al., 2009). Loss-of-function studies showed that miR-34a repression increased GRM7 mRNA levels, presumably by removing its negative repressor. Future studies aimed at understanding the mechanisms by which ncRNAs direct or influence the actions of current mood stabilizers will undoubtedly uncover new insights into the pathophysiology of mood disorders and may also yield novel drug targets.

9. Drugs of abuse

Drugs of abuse disrupt CNS development and trigger cellular and molecular changes in brain reward systems, resulting in long-lived structural and functional modifications that underlie tolerance, a phenomenon in which decreased responses to the same dose of drug occur over time. The counterbalance to the induction of tolerance is the formation of compensatory homeostatic plasticity wherein long-term drug exposure triggers an anti-addiction adaptive response in brain reward circuitries to ultimately reduce drug consumption. The following section highlights the emerging roles of miRNAs in mechanisms of developmental neurotoxicity, tolerance, and homeostatic plasticity.

9.1. Ethanol

In a fetal mouse cerebral cortex-derived culture model, exposure to ethanol increases markers of cell proliferation and depletes the neural epithelium of stem-cell populations by triggering premature neural stem-cell maturation (Santillano et al., 2005). A follow-up study identified a number of miRNAs, miR-21, -335, -9, and -153, whose expression were suppressed upon exposure to ethanol in fetal mouse cerebral cortical neuroepithelium (Sathyan et al., 2007). Among them, miR-21 and miR-335 were subsequently examined to probe the effects of miRNA repression by ethanol (Fig. 3 and Sathyan et al., 2007). GABA_A-receptor antagonism by picrotoxin blocked ethanol-induced declines in miR-21, but not miR-335 expression (Sathyan et al., 2007). Similar to other reports (Fig. 2, Chan et al., 2005), miR-21 acts as an anti-apoptotic factor whereby its suppression by ethanol increases apoptotic cell death in neural epithelium (Fig. 3, Sathyan et al., 2007). miR-335 knockdown revealed that the miRNA negatively regulates cell proliferation, and its repression by ethanol may be one mechanism by which the drug triggers an increase in cell proliferation and blocks the pro-apoptotic effects of miR-21 repression (Fig. 3). This study highlights the divergent effects of ethanol-sensitive miRNAs and illustrates how coordinately expressed miRNAs can exhibit functional antagonism toward one another (Fig. 6).

Early-life chemical exposures are increasingly associated with long-lasting effects in neurobehavioral function and development (Korosi et al., 2012). There are a handful of reports linking a specific miRNA with a functional behavioral phenotype *in vivo* (Kocerha et al., 2009; Tal et al., 2012). Kocerha and colleagues showed that repression of miR-219 expression in the pre-frontal cortex, following acute exposure to an NMDA receptor inhibitor, functions as a compensatory mechanism by which pharmacological locomotor manifestations were abrogated in adult mice (Kocerha et al., 2009). Similar to Sathyan et al.

(2007), we observed robust repression of miR-9 and miR-153c in zebrafish embryos transiently exposed to ethanol during early neurogenesis (Tal et al., 2012). In support of a role for ethanol-sensitive miRNAs in the development of the vertebrate neurobehavioral system, antisense-mediated knockdown of miR-9 and miR-153c or exposure to ethanol results in persistent neurobehavioral effects in larval and juvenile zebrafish (Tal et al., 2012). Together, these studies indicate that miRNAs may diminish or mediate drug-related behavioral effects and underscore the relevance of miRNA disruption during neurobehavioral development as a consequence of fetal exposure to drugs of abuse.

In addition to their proposed roles during neurobehavioral development, ethanol-sensitive miRNAs have been associated with tolerance. The neuronal large-conductance calcium-activated and voltage-gated potassium (BK) channel is highly expressed in the brain, influences neuronal excitability, transmitter release, and firing frequency and is a known modifier of ethanol sensitivity (Treistman and Martin, 2009). BK channels also exhibit tolerance to ethanol, or a decrease in receptor activity, as a result of sustained or repeated exposure to the drug (Pietrzykowski et al., 2008; Treistman and Martin, 2009). In support of a central role for the BK receptor in ethanol sensitivity and tolerance, deletion of the BK channel gene blocks ethanol-mediated behavioral responses and the development of tolerance (Cowmeadow et al., 2005; Davies et al., 2003). These results imply that post-transcriptional changes in channel number or subunit composition might contribute to ethanol tolerance. An elegant study by Pietrzykowski and colleagues showed that miR-9, upregulated upon exposure to ethanol, specifically targets a subset of BK channel splice variants that contain miR-9 MREs in their 3'-UTRs in rat supraoptic nucleus explants (Fig. 3, Pietrzykowski et al., 2008). The remaining splice variants encode BK isoforms with reduced responsiveness to ethanol. These data provide a strong mechanistic basis for miR-9-mediated neuroadaptation to ethanol, and more broadly, describe a mechanism for regulation of alternatively spliced mRNA by miRNAs relevant to both drug adaptation and neuronal plasticity. Because ethanol exposure both downregulates (Sathyan et al., 2007; Tal et al., 2012) and upregulates (Pietrzykowski et al., 2008) miR-9 expression in a context-dependent manner, we propose that miR-9 may function as an ethanol-sensitive miRNA switch depending on developmental stage or model system (Tal et al., 2012). More broadly, these studies illustrate a common theme in miRNA research: the same miRNA can exert multiple different developmental and cellular context-specific functions (Gao, 2010).

9.2. Opiates

Morphine is an opioid analgesic widely used to treat chronic pain. Chronic treatment with the drug produces tolerance via synaptic remodeling at the cellular level and changes in neuronal circuitry following activation of the μ -opioid receptor (Ueda and Ueda, 2009). Expression of the μ -opioid receptor is reportedly under the control of miRNAs (Wu et al., 2009). In mouse neuronal cells exposed to morphine, elevated levels of miR23b postranscriptionally target the receptor through a miRNA feedback circuit (Fig. 6 and Wu et al., 2009).

Similar to other G-protein-coupled receptors, agonists of the μ -opioid receptor produce unique physiological effects by activation of distinct downstream signaling pathways. In the case of the μ -opioid receptor agonists morphine and fentanyl, ERK1/2-dependent signaling involves activation of protein kinase C (PKC) or β -arrestin2, respectively (Zheng et al., 2010). Pathway-specific activation triggers unique suites of downstream signaling events including the expression of disparate profiles of miRNAs (Zheng et al., 2010). In primary hippocampal neurons and *in vivo*, in excised hippocampal and cerebellar tissue, exposure to fentanyl reduced miR-184, -190, and -301 levels while miR-20a expression was specifically repressed by morphine (Zheng et al., 2010). miR-190 repression by fentanyl is μ -opioid receptor, β -arrestin2-, and ERK1/2-dependent (Zheng et al., 2010). Repression of miR-133b

expression upon exposure to morphine is similarly downstream to ERK1/2-dependent signaling (Sanchez-Simon et al., 2010). In both embryonic zebrafish and immature rat hippocampal neurons, but not mature neurons, morphine exposure reduces levels of miR-133b (Sanchez-Simon et al., 2010), a miRNA that is reduced in the brains of Parkinson's patients (Kim et al., 2007). Collectively, these studies expand the current body of literature describing agonist-specific consequences of GPCR activity to include miRNAs.

9.3. Cocaine

Extended access to drugs of abuse, including cocaine, typically results in increased motivation to consume the drug. Intriguingly, in dopaminergic neurons, loss of argonaute 2 (AGO2), a protein involved in miRNA biogenesis and in the execution of miRNA-dependent silencing, markedly reduces motivation for cocaine self-administration in mice (Schaefer et al., 2010). miRNAs are also associated with counter-adaptive homeostatic plasticity. Expression profiling performed in the dorsal striatum, a key brain region involved in compulsive cocaine use, identified increased levels of miR-212 in rats with unrestricted access to intravenous cocaine (Hollander et al., 2010). Using a lentiviral delivery approach, Hollander et al. showed that under conditions of unrestricted access to cocaine, striatal overexpression of miR-212 markedly and progressively reduced cocaine intake relative to control animals injected with empty vector (Hollander et al., 2010). This effect was not observed in rats with elevated striatal miR-212 levels allowed only controlled access to the drug (Hollander et al., 2010). Further, striatal knockdown of miR-212 *in vivo* increased cocaine consumption (Hollander et al., 2010). Mechanistically, cocaine stimulates CREB-dependent miR-212 expression that acts in a feed-forward fashion to amplify the CREB-TOR signaling cascade, in part through repression of the miR-212 target SPRED1 (Fig. 4 and Hollander et al., 2010). A subsequent study showed that miR-212 additionally regulates cocaine intake via a negative homeostatic interaction with MeCP2, a transcriptional repressor that is post-transcriptionally regulated by miR-212 and that in turn, represses miR-212 expression (Fig. 4 and Im et al., 2010). Taken together, these studies represent a previously unknown mechanism of counter-adaption to cocaine overconsumption and suggest that reduced miR-212 levels may increase vulnerability to addiction. It remains to be seen whether drugs targeting miRNAs such as miR-212 can be employed to combat addiction by replicating or blocking the actions of miRNAs involved in homeostatic plasticity or tolerance.

9.4. Nicotine

The GTPase Dynamin 1 (Dnm1) is significantly associated with nicotine dependence in human smokers (Xu et al., 2009) and rats (Xu and Li, 2011). In neurons, Dnm1 influences synaptic function by mediating late stages of endocytosis (Smillie and Cousin, 2005). In PC12 cells, upregulation of miR140* in response to nicotine exposure is coordinately regulated with wwp2 expression, a gene whose intron encodes the miR140/140* genes (Huang and Li, 2009). Application of exogenous miR-140* depleted dnm1 mRNA levels and reduced luciferase activity in cells transfected with a reporter construct containing the dnm1 3'-UTR (Huang and Li, 2009). In the converse experiment, miR-140* knockdown elevated luciferase activity and the abundance of endogenous dnm1 transcripts (Huang and Li, 2009). Finally, mutation of the two putative miR-140* binding sites in the dnm1 3'-UTR abolished the ability of the miRNA to target dnm1 transcripts (Huang and Li, 2009). While more experiments are necessary to determine whether dnm1 is subject to regulation by miR-140* under conditions of nicotine exposure, these data show that miR-140* post-transcriptionally regulates dnm1 and are suggestive of a novel mechanism by which nicotine may modulate synaptic function. Exposure to nicotine or cocaine also upregulates levels of miR-29a and miR-29b in multiple mouse brain regions and in primary mouse hippocampal neurons (Lippi et al., 2011). These activity-dependent miRNA function as negative regulators

of dendritic spine formation by post-transcriptionally repressing levels of the Arp3 actin nucleation factor, a molecule that drives spine head enlargement and synaptogenesis (Lippi et al., 2011). The same study showed that levels of miR-29a and miR-29b are also elevated in the hippocampus during contextual fear learning (Lippi et al., 2011) suggesting that miRNAs that regulate spine remodeling under physiological conditions may also mediate drug-induced neuroplasticity.

10. miRNA-mediated neurotoxicity of chemicals in the environment

Relative to pharmacological agents and drugs of abuse, there is a paucity of data describing mechanisms by which environmentally relevant compounds elicit toxicity by deregulating miRNA-dependent signaling. The following section highlights the emerging role of miRNAs as effectors of environmental neurotoxins and the roles that some play choreographing adaptive cellular responses to chemical exposures. In general, a considerable amount of work is needed to understand whether alterations in miRNA expression are causal or merely associated with adverse neurological outcomes.

10.1. Metals

Aluminum (Al) is a ubiquitous neurotoxic metal. Exposure to aluminum-salts accelerates brain aging, in part through the promotion of neuronal inflammation, and is considered to be an environmental factor involved in the pathogenesis of Alzheimer's disease (AD, reviewed in Bondy, 2010). AD is clinically characterized by the progressive erosion of cognition and memory and an up-regulation of inflammatory signaling. More recently, differences in the expression of a number of miRNAs have been detected in human neuronal cells (Lukiw and Pogue, 2007) and human AD brains (Nelson and Wang, 2010; Sethi and Lukiw, 2009), including the upregulation of miR-146a, a post-transcriptional regulator of the inflammatory repressor complement factor H (Lukiw et al., 2008). In a co-culture of human neuronal and glial cells (HN cells), Pogue and colleagues showed that aluminum-sulfate exposure elevates miR-146a expression, a gene with three nuclear factor kappa beta (NF- κ B) binding sites in its upstream promoter (Pogue et al., 2009). Elevated expression of miR-146a and miR-125b, another NF- κ B sensitive miRNA, was reported in human astroglial cells exposed to iron and aluminum (Pogue et al., 2011). In support of a metal-stress responsive role for miR-146a, aluminum-sulfate exposure upregulated NF- κ B, generated reactive oxygen species (ROS), and upregulated miR-146a promoter-driven expression of a reporter gene in HN cells (Fig. 5 and Pogue et al., 2009). Downregulation of the inflammatory repressor complement factor H was additionally reported upon exposure to the metal-sulfate (Pogue et al., 2009). Treatment with amyloid beta 42 (A β 42) peptide also upregulated miR-146a promoter-reporter expression and decreased complement factor H levels (Fig. 5 and Pogue et al., 2009). Taken together, the authors suggest that aluminum-sulfate exposure may influence the pathogenesis of AD via NF- κ B-mediated upregulation of miR-146a and subsequent repression of complement factor H transcript levels (Pogue et al., 2009). While, further gain- and loss-of-function studies are needed to fully elucidate causal relationships between NF- κ B, miR-146a, and complement factor H, this study provides a tantalizing example of how blocking anti-inflammatory signaling may elicit neurotoxicity. It will be interesting to determine whether other compounds that evoke neurotoxicity via oxidative stress also involve the upregulation of miR-146a and other NF κ B-sensitive miRNAs.

10.2. Pesticides

To date, a single report has examined the effect of pesticides on miRNA expression. Wang et al. investigated the effects of fipronil, a member of the phenylpyrazole class of pesticides, and the organophosphate pesticide triazophos on miRNA expression in adult zebrafish (Wang et al., 2010). Differential expression of 14 and 3 miRNAs was detected following

exposure to triazophos or fipronil, respectively (Wang et al., 2010). More work is needed to understand the biological relevance of these findings. This can be achieved by linking misexpression of specific miRNAs to adverse phenotypic outcomes as well as by identifying the full repertoire of downstream targets (and their phenotypic relevance) post-transcriptionally regulated by the miRNAs in question.

10.3. RDX

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a trinitrated cyclic compound used as an explosive. Environmental contamination can occur during the manufacture, use, and disposal of RDX-containing munitions and can leach into groundwater from unexploded munitions located on training ranges. RDX exposure triggers seizures by binding to the picrotoxin convulsant site of the GABA_A receptor (Williams et al., 2011). In a chronic feeding study, Zhang and Pan reported that mice exposed to RDX present with alterations in brain and liver miRNA expression profiles (Zhang and Pan, 2009). A modest number of miRNAs were similarly up- or downregulated in both brain and liver samples. The majority of changes however, were specific to brain (50%) or liver (26%) tissue (Zhang and Pan, 2009). The most highly induced miRNA, miR-206, is predicted to post-transcriptionally regulate multiple splice variants of the brain derived neurotrophic factor (BDNF), a gene that is downregulated in a number of neurodegenerative diseases (Murer et al., 2001). Since two bona fide regulators of BDNF transcript levels, miR-30a-5p and miR-195 (Mellios et al., 2008), were also upregulated in their dataset, the authors speculate that RDX exposure may disrupt BDNF-dependent signaling (Zhang and Pan, 2009), but more work is needed to confirm this. Again, if unanchored to a phenotypic outcome or to the repression of specific mRNA transcripts, the relevance of miRNA misexpression upon chemical exposure will remain unclear.

11. Concluding remarks

The studies described here illustrate our emerging understanding of the role of ncRNAs as novel players in neurotoxicology. While there is certainly increasing interest in ncRNAs in the field, more research is needed to fully elucidate the mechanisms by which ncRNAs modulate drug or toxicant modes of action (Fig. 6). This will require us to eschew changes in ncRNA expression as the sole indicator by which miRNA dysregulation is purported to exert neurotoxicity. The continual discovery of new members and classes of ncRNAs and the overwhelmingly undefined repertoire of ncRNA targets that shift with developmental stage, tissue, or cell type, certainly render the system difficult to comprehend. Yet despite the vast and ever-shifting ncRNA landscape, it is critical for the field to wade through the complexity of global changes in ncRNA expression in order to distinguish ncRNAs that are toxicologically relevant from those whose changes in expression are merely correlated with chemical exposures (Fig. 6). This will require a more precise understanding of downstream ncRNA-targets that are firmly anchored to toxicological phenotypes. In addition, studies examining initiating molecular events, including altered biogenesis, transcription, or ncRNA stability, that precipitate changes in ncRNA levels are necessary to fully elucidate the mechanisms by which ncRNA drive or modify toxicity (Fig. 6). In addition, because ncRNAs often act in concert with one another, it is also critical to explore the potential synergism or functional antagonism of other coordinately regulated ncRNAs in a toxicological context (Fig. 6). It remains poorly understood whether ncRNAs function to compensate for the adverse effects of neurotoxicant exposure or what role they may play in toxicological feed-forward or feedback circuitry (Fig. 6). The majority of studies discussed here focus on disruptions to the miRNA ncRNA system. More work is needed to clarify the involvement of other types of ncRNAs in mechanisms of neurotoxicity.

Acknowledgments

We regret that space constraints have prevented the citation of many relevant studies. This work was supported by the NIEHS Environmental Health Sciences Core Center Grant ES00210, NIEHS Training Grant T32ES07060, and a Superfund Basic Research Program Grant NIEHS P42 ES016465 to RLT. We are grateful to Sue Edelstein for help preparing graphics and thank Jill Franzosa, Britton Goodale, Windy Boyd, and Julie Hall for their helpful discussions and critical review of the manuscript.

References

- Alliey-Rodriguez N, Zhang D, Badner JA, Lahey BB, Zhang X, Dinwiddie S, et al. Genome-wide association study of personality traits in bipolar patients. *Psychiatr Genet*. 2011; 21:190–4. [PubMed: 21368711]
- Aravin AA, Hannon GJ, Brennecke J. The Piwi-piRNA pathway provides an adaptive defense in the transposon arms race. *Science*. 2007; 318:761–4. [PubMed: 17975059]
- Au PC, Zhu QH, Dennis ES, Wang MB. Long non-coding RNA-mediated mechanisms independent of the RNAi pathway in animals and plants. *RNA Biol*. 2011; 8:404–14. [PubMed: 21525783]
- Barbee SA, Estes PS, Cziko AM, Hillebrand J, Luedeman RA, Collier JM, et al. Staufen- and FMRP-containing neuronal RNPs are structurally and functionally related to somatic P bodies. *Neuron*. 2006; 52:997–1009. [PubMed: 17178403]
- Baudry A, Mouillet-Richard S, Schneider B, Launay JM, Kellermann O. miR-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. *Science*. 2010; 329:1537–41. [PubMed: 20847275]
- Bejerano G, Pheasant M, Makunin I, Stephen S, Kent WJ, Mattick JS, et al. Ultraconserved elements in the human genome. *Science*. 2004; 304:1321–5. [PubMed: 15131266]
- Benmansour S, Owens WA, Cecchi M, Morilak DA, Frazer A. Serotonin clearance *in vivo* is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. *J Neurosci*. 2002; 22:6766–72. [PubMed: 12151556]
- Bondy SC. The neurotoxicity of environmental aluminum is still an issue. *Neurotoxicology*. 2010; 31:575–81. [PubMed: 20553758]
- Bourne JN, Harris KM. Balancing structure and function at hippocampal dendritic spines. *Annu Rev Neurosci*. 2008; 31:47–67. [PubMed: 18284372]
- Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. *PLoS Biol*. 2005; 3:e85. [PubMed: 15723116]
- Chan JA, Krichevsky AM, Kosik KS. MicroRNA-21 is an antiapoptotic factor in human glioblastoma cells. *Cancer Res*. 2005; 65:6029–33. [PubMed: 16024602]
- Chatterjee S, Grosshans H. Active turnover modulates mature microRNA activity in *Caenorhabditis elegans*. *Nature*. 2009; 461:546–9. [PubMed: 19734881]
- Corcoran DL, Pandit KV, Gordon B, Bhattacharjee A, Kaminski N, Benos PV. Features of mammalian microRNA promoters emerge from polymerase II chromatin immunoprecipitation data. *PloS One*. 2009; 4:e5279. [PubMed: 19390574]
- Cougot N, Bhattacharyya SN, Tapia-Arancibia L, Bordonne R, Filipowicz W, Bertrand E, et al. Dendrites of mammalian neurons contain specialized P-body-like structures that respond to neuronal activation. *J Neurosci*. 2008; 28:13793–804. [PubMed: 19091970]
- Cowmeadow RB, Krishnan HR, Atkinson NS. The slowpoke gene is necessary for rapid ethanol tolerance in *Drosophila*. *Alcohol Clin Exp Res*. 2005; 29:1777–86. [PubMed: 16269907]
- Davies AG, Pierce-Shimomura JT, Kim H, VanHoven MK, Thiele TR, Bonci A, et al. A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell*. 2003; 115:655–66. [PubMed: 14675531]
- Davis TH, Cuellar TL, Koch SM, Barker AJ, Harfe BD, McManus MT, et al. Conditional loss of Dicer disrupts cellular and tissue morphogenesis in the cortex and hippocampus. *J Neurosci*. 2008; 28:4322–30. [PubMed: 18434510]
- De Pietri Tonelli D, Pulvers JN, Haffner C, Murchison EP, Hannon GJ, Huttner WB. miRNAs are essential for survival and differentiation of newborn neurons but not for expansion of neural

- progenitors during early neurogenesis in the mouse embryonic neocortex. *Development*. 2008; 135:3911–21. [PubMed: 18997113]
- Derrien T, Guigo R, Johnson R. The long non-coding RNAs: a new (P)layer in the dark matter. *Front Genet*. 2011; 2:107. [PubMed: 22303401]
- Du T, Zamore PD. microPrimer: the biogenesis and function of microRNA. *Development*. 2005; 132:4645–52. [PubMed: 16224044]
- Esteller M. Non-coding RNAs in human disease. *Nat Rev Genet*. 2011; 12:861–74. [PubMed: 22094949]
- Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem*. 2010; 79:351–79. [PubMed: 20533884]
- Faghihi MA, Modarresi F, Khalil AM, Wood DE, Sahagan BG, Morgan TE, et al. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. *Nat Med*. 2008; 14:723–30. [PubMed: 18587408]
- Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res*. 2009; 19:92–105. [PubMed: 18955434]
- Gao FB. Context-dependent functions of specific microRNAs in neuronal development. *Neural Dev*. 2010; 5:25. [PubMed: 20920300]
- Gehrke S, Imai Y, Sokol N, Lu B. Pathogenic LRRK2 negatively regulates microRNA-mediated translational repression. *Nature*. 2010; 466:637–41. [PubMed: 20671708]
- Ghildiyal M, Zamore PD. Small silencing RNAs: an expanding universe. *Nat Rev Genet*. 2009; 10:94–108. [PubMed: 19148191]
- Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, et al. MicroRNAs regulate brain morphogenesis in zebrafish. *Science*. 2005; 308:833–8. [PubMed: 15774722]
- Gopinath SC, Matsugami A, Katahira M, Kumar PK. Human vault-associated non-coding RNAs bind to mitoxantrone, a chemotherapeutic compound. *Nucleic Acids Res*. 2005; 33:4874–81. [PubMed: 16150923]
- Gopinath SC, Wadhwa R, Kumar PK. Expression of noncoding vault RNA in human malignant cells and its importance in mitoxantrone resistance. *Mol Cancer Res*. 2010; 8:1536–46. [PubMed: 20881010]
- Grosshans H, Chatterjee S. MicroRNAs and the regulated degradation of mature animal miRNAs. *Adv Exp Med Biol*. 2010; 700:140–55.
- Guengerich FP. Cytochrome p450 and chemical toxicology. *Chem Res Toxicol*. 2008; 21:70–83. [PubMed: 18052394]
- Hamilton AJ, Baulcombe DC. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science*. 1999; 286:950–2. [PubMed: 10542148]
- Hansen KF, Sakamoto K, Wayman GA, Impey S, Obrietan K. Transgenic miR132 alters neuronal spine density and impairs novel object recognition memory. *PloS One*. 2010; 5:e15497. [PubMed: 21124738]
- Hebert SS, Papadopoulou AS, Smith P, Galas MC, Planel E, Silahtaroglu AN, et al. Genetic ablation of Dicer in adult forebrain neurons results in abnormal tau hyperphosphorylation and neurodegeneration. *Hum Mol Genet*. 2010; 19:3959–69. [PubMed: 20660113]
- Hinske LC, Galante PA, Kuo WP, Ohno-Machado L. A potential role for intragenic miRNAs on their hosts' interactome. *BMC Genomics*. 2010; 11:533. [PubMed: 20920310]
- Hollander JA, Im HI, Amelio AL, Kocerha J, Bali P, Lu Q, et al. Striatal microRNA controls cocaine intake through CREB signalling. *Nature*. 2010; 466:197–202. [PubMed: 20613834]
- Huang T, Liu Y, Huang M, Zhao X, Cheng L. Wnt1-cre-mediated conditional loss of Dicer results in malformation of the midbrain and cerebellum and failure of neural crest and dopaminergic differentiation in mice. *J Mol Cell Biol*. 2010; 2:152–63. [PubMed: 20457670]
- Huang W, Li MD. Nicotine modulates expression of miR-140*, which targets the 3'-untranslated region of dynamin 1 gene (Dnm1). *Int J Neuropsychopharmacol*. 2009; 12:537–46. [PubMed: 18845019]

- Im HI, Hollander JA, Bali P, Kenny PJ. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. *Nat Neurosci.* 2010; 13:1120–7. [PubMed: 20711185]
- Impey S, Davare M, Lasiek A, Fortin D, Ando H, Varlamova O, et al. An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. *Mol Cell Neurosci.* 2010; 43:146–56. [PubMed: 19850129]
- Jacobsen A, Wen J, Marks DS, Krogh A. Signatures of RNA binding proteins globally coupled to effective microRNA target sites. *Genome Res.* 2010; 20:1010–9. [PubMed: 20508147]
- Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, et al. RNA maps reveal new RNA classes and a possible function for pervasive transcription. *Science.* 2007; 316:1484–8. [PubMed: 17510325]
- Kapsimali M, Kloosterman WP, de Bruijn E, Rosa F, Plasterk RH, Wilson SW. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol.* 2007; 8:R173. [PubMed: 17711588]
- Kawashima H, Numakawa T, Kumamaru E, Adachi N, Mizuno H, Ninomiya M, et al. Glucocorticoid attenuates brain-derived neurotrophic factor-dependent upregulation of glutamate receptors via the suppression of microRNA-132 expression. *Neuroscience.* 2010; 165:1301–11. [PubMed: 19958814]
- Kim J, Inoue K, Ishii J, Vanti WB, Voronov SV, Murchison E, et al. A MicroRNA feedback circuit in midbrain dopamine neurons. *Science.* 2007; 317:1220–4. [PubMed: 17761882]
- Kim VN. MicroRNA biogenesis: coordinated cropping and dicing. *Nat Rev Mol Cell Biol.* 2005; 6:376–85. [PubMed: 15852042]
- Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol.* 2009; 10:126–39. [PubMed: 19165215]
- Kiss-Laszlo Z, Henry Y, Bachellerie JP, Caizergues-Ferrer M, Kiss T. Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. *Cell.* 1996; 85:1077–88. [PubMed: 8674114]
- Klann E, Dever TE. Biochemical mechanisms for translational regulation in synaptic plasticity. *Nat Rev Neurosci.* 2004; 5:931–42. [PubMed: 15550948]
- Kocerha J, Faghihi MA, Lopez-Toledano MA, Huang J, Ramsey AJ, Caron MG, et al. MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction. *Proc Natl Acad Sci U S A.* 2009; 106:3507–12. [PubMed: 19196972]
- Korosi A, Naninck EF, Oomen CA, Schouten M, Krugers H, Fitzsimons C, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. *Behav Brain Res.* 2012; 227:400–9. [PubMed: 21821065]
- Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS. A microRNA array reveals extensive regulation of microRNAs during brain development. *RNA.* 2003; 9:1274–81. [PubMed: 13130141]
- Krol J, Busskamp V, Markiewicz I, Stadler MB, Ribi S, Richter J, et al. Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. *Cell.* 2010a; 141:618–31. [PubMed: 20478254]
- Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet.* 2010b; 11:597–610. [PubMed: 20661255]
- Kumamaru E, Numakawa T, Adachi N, Yagasaki Y, Izumi A, Niyaz M, et al. Glucocorticoid prevents brain-derived neurotrophic factor-mediated maturation of synaptic function in developing hippocampal neurons through reduction in the activity of mitogen-activated protein kinase. *Mol Endocrinol.* 2008; 22:546–58. [PubMed: 18096693]
- Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T. Identification of novel genes coding for small expressed RNAs. *Science.* 2001; 294:853–8. [PubMed: 11679670]
- Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. *Science.* 2001; 294:858–62. [PubMed: 11679671]
- Lau P, de Strooper B. Dysregulated microRNAs in neurodegenerative disorders. *Semin Cell Dev Biol.* 2010; 21:768–73. [PubMed: 20080199]

- Lee RC, Ambros V. An extensive class of small RNAs in *Caenorhabditis elegans*. *Science*. 2001; 294:862–4. [PubMed: 11679672]
- Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, et al. The nuclear RNase III Drosha initiates microRNA processing. *Nature*. 2003; 425:415–9. [PubMed: 14508493]
- Li X, Jin P. Macro role(s) of microRNAs in fragile X syndrome? *Neuromolecular Med*. 2009; 11:200–7. [PubMed: 19669947]
- Ling SC, Albuquerque CP, Han JS, Lagier-Tourenne C, Tokunaga S, Zhou H, et al. ALS-associated mutations in TDP-43 increase its stability and promote TDP-43 complexes with FUS/TLS. *Proc Natl Acad Sci U S A*. 2010; 107:13318–23. [PubMed: 20624952]
- Lippi G, Steinert JR, Marczylo EL, D'Oro S, Fiore R, Forsythe ID, et al. Targeting of the Arpc3 actin nucleation factor by miR-29a/b regulates dendritic spine morphology. *J Cell Biol*. 2011; 194:889–904. [PubMed: 21930776]
- Lugli G, Larson J, Martone ME, Jones Y, Smalheiser NR. Dicer and eIF2c are enriched at postsynaptic densities in adult mouse brain and are modified by neuronal activity in a calpain-dependent manner. *J Neurochem*. 2005; 94:896–905. [PubMed: 16092937]
- Lukiw WJ, Cui JG, Yuan LY, Bhattacharjee PS, Corkern M, Clement C, et al. Acyclovir or Abeta42 peptides attenuate HSV-1-induced miRNA-146a levels in human primary brain cells. *Neuroreport*. 2010; 21:922–7. [PubMed: 20683212]
- Lukiw WJ, Pogue AI. Induction of specific micro RNA (miRNA) species by ROS-generating metal sulfates in primary human brain cells. *J Inorg Biochem*. 2007; 101:1265–9. [PubMed: 17629564]
- Lukiw WJ, Zhao Y, Cui JG. An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. *J Biol Chem*. 2008; 283:31315–22. [PubMed: 18801740]
- Ma J, Murphy M, O'Dwyer PJ, Berman E, Reed K, Gallo JM. Biochemical changes associated with a multidrug-resistant phenotype of a human glioma cell line with temozolomide-acquired resistance. *Biochem Pharmacol*. 2002; 63:1219–28. [PubMed: 11960598]
- Magill ST, Cambronne XA, Luikart BW, Lioy DT, Leighton BH, Westbrook GL, et al. microRNA-132 regulates dendritic growth and arborization of newborn neurons in the adult hippocampus. *Proc Natl Acad Sci U S A*. 2010; 107:20382–7. [PubMed: 21059906]
- Mattick JS. RNA regulation: a new genetics? *Nat Rev Genet*. 2004; 5:316–23. [PubMed: 15131654]
- Mellios N, Huang HS, Grigorenko A, Rogaev E, Akbarian S. A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. *Hum Mol Genet*. 2008; 17:3030–42. [PubMed: 18632683]
- Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, et al. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology*. 2006; 130:2113–29. [PubMed: 16762633]
- Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet*. 2009; 10:155–9. [PubMed: 19188922]
- Miller TE, Ghoshal K, Ramaswamy B, Roy S, Datta J, Shapiro CL, et al. MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. *J Biol Chem*. 2008; 283:29897–903. [PubMed: 18708351]
- Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog Neurobiol*. 2001; 63:71–124. [PubMed: 11040419]
- Nakajima M, Yokoi T. MicroRNAs from biology to future pharmacotherapy: regulation of cytochrome P450s and nuclear receptors. *Pharmacol Ther*. 2011
- Nelson PT, Wang WX. MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study. *J Alzheimers Dis*. 2010; 21:75–9. [PubMed: 20413881]
- Pan YZ, Gao W, Yu AM. MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. *Drug Metab Dispos*. 2009; 37:2112–7. [PubMed: 19581388]
- Parker R, Sheth U. P bodies and the control of mRNA translation and degradation. *Mol Cell*. 2007; 25:635–46. [PubMed: 17349952]

- Persson H, Kvist A, Vallon-Christersson J, Medstrand P, Borg A, Rovira C. The non-coding RNA of the multidrug resistance-linked vault particle encodes multiple regulatory small RNAs. *Nat Cell Biol.* 2009; 11:1268–71. [PubMed: 19749744]
- Pietrzykowski AZ, Friesen RM, Martin GE, Puig SI, Nowak CL, Wynne PM, et al. Posttranscriptional regulation of BK channel splice variant stability by miR-9 underlies neuroadaptation to alcohol. *Neuron.* 2008; 59:274–87. [PubMed: 18667155]
- Pinter R, Hindges R. Perturbations of microRNA function in mouse dicer mutants produce retinal defects and lead to aberrant axon pathfinding at the optic chiasm. *PloS One.* 2010; 5:e10021. [PubMed: 20386732]
- Pogue AI, Li YY, Cui JG, Zhao Y, Kruck TP, Percy ME, et al. Characterization of an NF-kappaB-regulated, miRNA-146a-mediated down-regulation of complement factor H (CFH) in metal-sulfate-stressed human brain cells. *J Inorg Biochem.* 2009; 103:1591–5. [PubMed: 19540598]
- Pogue AI, Percy ME, Cui JG, Li YY, Bhattacharjee S, Hill JM, et al. Up-regulation of NF-kB-sensitive miRNA-125b and miRNA-146a in metal sulfate-stressed human astroglial (HAG) primary cell cultures. *J Inorg Biochem.* 2011; 105:1434–7. [PubMed: 22099153]
- Preker R, Nielsen J, Kammler S, Lykke-Andersen S, Christensen MS, Mapendano CK, et al. RNA exosome depletion reveals transcription upstream of active human promoters. *Science.* 2008; 322:1851–4. [PubMed: 19056938]
- Remenyi J, Hunter CJ, Cole C, Ando H, Impey S, Monk CE, et al. Regulation of the miR-212/132 locus by MSK1 and CREB in response to neurotrophins. *Biochem J.* 2010; 428:281–91. [PubMed: 20307261]
- Ren Y, Zhou X, Mei M, Yuan XB, Han L, Wang GX, et al. MicroRNA-21 inhibitor sensitizes human glioblastoma cells U251 (PTEN-mutant) and LN229 (PTEN-wild type) to taxol. *BMC Cancer.* 2010; 10:27. [PubMed: 20113523]
- Rom S, Rom I, Passiatore G, Pacifici M, Radhakrishnan S, Del Valle L, et al. CCL8/MCP-2 is a target for mir-146a in HIV-1-infected human microglial cells. *FASEB J.* 2010; 24:2292–300. [PubMed: 20181935]
- Roos WP, Batista LF, Naumann SC, Wick W, Weller M, Menck CF, et al. Apoptosis in malignant glioma cells triggered by the temozolomide-induced DNA lesion O6-methylguanine. *Oncogene.* 2007; 26:186–97. [PubMed: 16819506]
- Sanchez-Simon FM, Zhang XX, Loh HH, Law PY, Rodriguez RE. Morphine regulates dopaminergic neuron differentiation via miR-133b. *Mol Pharmacol.* 2010; 78:935–42. [PubMed: 20716624]
- Santillano DR, Kumar LS, Prock TL, Camarillo C, Tingling JD, Miranda RC. Ethanol induces cell-cycle activity and reduces stem cell diversity to alter both regenerative capacity and differentiation potential of cerebral cortical neuroepithelial precursors. *BMC Neurosci.* 2005; 6:59. [PubMed: 16159388]
- Sathyan P, Golden HB, Miranda RC. Competing interactions between micro-RNAs determine neural progenitor survival and proliferation after ethanol exposure: evidence from an ex vivo model of the fetal cerebral cortical neuroepithelium. *J Neurosci.* 2007; 27:8546–57. [PubMed: 17687032]
- Schaefer A, Im HI, Veno MT, Fowler CD, Min A, Intrator A, et al. Argonaute 2 in dopamine 2 receptor-expressing neurons regulates cocaine addiction. *J Exp Med.* 2010; 207:1843–51. [PubMed: 20643829]
- Schaefer A, O'Carroll D, Tan CL, Hillman D, Sugimori M, Llinas R, et al. Cerebellar neurodegeneration in the absence of microRNAs. *J Exp Med.* 2007; 204:1553–8. [PubMed: 17606634]
- Schratt GM, Tuebing F, Nigh EA, Kane CG, Sabatini ME, Kiebler M, et al. A brain-specific microRNA regulates dendritic spine development. *Nature.* 2006; 439:283–9. [PubMed: 16421561]
- Seila AC, Calabrese JM, Levine SS, Yeo GW, Rahl PB, Flynn RA, et al. Divergent transcription from active promoters. *Science.* 2008; 322:1849–51. [PubMed: 19056940]
- Sethi P, Lukiw WJ. Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. *Neurosci Lett.* 2009; 459:100–4. [PubMed: 19406203]

- Shi L, Chen J, Yang J, Pan T, Zhang S, Wang Z. MiR-21 protected human glioblastoma U87MG cells from chemotherapeutic drug temozolomide induced apoptosis by decreasing Bax/Bcl-2 ratio and caspase-3 activity. *Brain Res.* 2010; 1352:255–64. [PubMed: 20633539]
- Siegel G, Obernosterer G, Fiore R, Oehmen M, Bicker S, Christensen M, et al. A functional screen implicates microRNA-138-dependent regulation of the depalmitoylation enzyme APT1 in dendritic spine morphogenesis. *Nat Cell Biol.* 2009; 11:705–16. [PubMed: 19465924]
- Skalsky RL, Cullen BR. Viruses, microRNAs, and host interactions. *Annu Rev Microbiol.* 2010; 64:123–41. [PubMed: 20477536]
- Smalheiser NR, Lugli G. microRNA regulation of synaptic plasticity. *Neuromolecular Med.* 2009; 11:133–40. [PubMed: 19458942]
- Smillie KJ, Cousin MA. Dynamin I phosphorylation and the control of synaptic vesicle endocytosis. *Biochem Soc Symp.* 2005:87–97. [PubMed: 15649133]
- Stark KL, Xu B, Bagchi A, Lai WS, Liu H, Hsu R, et al. Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. *Nat Genet.* 2008; 40:751–60. [PubMed: 18469815]
- Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S, Miyazono K. Modulation of microRNA processing by p53. *Nature.* 2009; 460:529–33. [PubMed: 19626115]
- Taft RJ, Glazov EA, Cloonan N, Simons C, Stephen S, Faulkner GJ, et al. Tiny RNAs associated with transcription start sites in animals. *Nat Genet.* 2009; 41:572–8. [PubMed: 19377478]
- Taft RJ, Pheasant M, Mattick JS. The relationship between non-protein-coding DNA and eukaryotic complexity. *Bioessays.* 2007; 29:288–99. [PubMed: 17295292]
- Takagi S, Nakajima M, Mohri T, Yokoi T. Post-transcriptional regulation of human pregnane X receptor by micro-RNA affects the expression of cytochrome P450 3A4. *J Biol Chem.* 2008; 283:9674–80. [PubMed: 18268015]
- Tal TL, Franzosa JA, Tilton SC, Philbrick KA, Iwaniec UT, Turner RT, et al. MicroRNAs control neurobehavioral development and function in zebrafish. *FASEB J.* 2012
- Treisman SN, Martin GE. BK Channels: mediators and models for alcohol tolerance. *Trends Neurosci.* 2009; 32:629–37. [PubMed: 19781792]
- Tsang J, Zhu J, van Oudenaarden A. MicroRNA-mediated feedback and feedforward loops are recurrent network motifs in mammals. *Mol Cell.* 2007; 26:753–67. [PubMed: 17560377]
- Ueda H, Ueda M. Mechanisms underlying morphine analgesic tolerance and dependence. *Front Biosci.* 2009; 14:5260–72. [PubMed: 19482614]
- Umbach JL, Kramer MF, Jurak I, Karnowski HW, Coen DM, Cullen BR. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature.* 2008; 454:780–3. [PubMed: 18596690]
- Vo N, Klein ME, Varlamova O, Keller DM, Yamamoto T, Goodman RH, et al. A cAMP-response element binding protein-induced microRNA regulates neuronal morphogenesis. *Proc Natl Acad Sci U S A.* 2005; 102:16426–31. [PubMed: 16260724]
- Vreugdenhil E, Verissimo CS, Mariman R, Kamphorst JT, Barbosa JS, Zweers T, et al. MicroRNA 18 and 124a down-regulate the glucocorticoid receptor: implications for glucocorticoid responsiveness in the brain. *Endocrinology.* 2009; 150:2220–8. [PubMed: 19131573]
- Wang X, Zhou S, Ding X, Zhu G, Guo J. Effect of triazophos, fipronil and their mixture on miRNA expression in adult zebrafish. *J Environ Sci Health B.* 2010; 45:648–57. [PubMed: 20818518]
- Wayman GA, Davare M, Ando H, Fortin D, Varlamova O, Cheng HY, et al. An activity-regulated microRNA controls dendritic plasticity by down-regulating p250GAP. *Proc Natl Acad Sci U S A.* 2008; 105:9093–8. [PubMed: 18577589]
- Weeraratne SD, Amani V, Neiss A, Teider N, Scott DK, Pomeroy SL, et al. miR-34a confers chemosensitivity through modulation of MAGE-A and p53 in medulloblastoma. *Neuro Oncol.* 2011; 13:165–75. [PubMed: 21177782]
- Williams LR, Aroniadou-Anderjaska V, Qashu F, Finne H, Pidoplichko V, Bannon DI, et al. RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RDX-induced seizures. *Environ Health Perspect.* 2011; 119:357–63. [PubMed: 21362589]

- Wu Q, Zhang L, Law PY, Wei LN, Loh HH. Long-term morphine treatment decreases the association of mu-opioid receptor (MOR1) mRNA with polysomes through miRNA23b. *Mol Pharmacol*. 2009; 75:744–50. [PubMed: 19144786]
- Wu X, Song Y. Preferential regulation of miRNA targets by environmental chemicals in the human genome. *BMC Genomics*. 2011; 12:244. [PubMed: 21592377]
- Xu Q, Huang W, Payne TJ, Ma JZ, Li MD. Detection of genetic association and a functional polymorphism of dynamin 1 gene with nicotine dependence in European and African Americans. *Neuropsychopharmacology*. 2009; 34:1351–9. [PubMed: 18987626]
- Xu Q, Li MD. Nicotine modulates expression of dynamin 1 in rat brain and SH-SY5Y cells. *Neurosci Lett*. 2011; 489:168–71. [PubMed: 21159320]
- Yu AM. Role of microRNAs in the regulation of drug metabolism and disposition. *Expert Opin Drug Metab Toxicol*. 2009; 5:1513–28. [PubMed: 19785514]
- Zehir A, Hua LL, Maska EL, Morikawa Y, Cserjesi P. Dicer is required for survival of differentiating neural crest cells. *Dev Biol*. 2010; 340:459–67. [PubMed: 20144605]
- Zhang B, Pan X. RDX induces aberrant expression of microRNAs in mouse brain and liver. *Environ Health Perspect*. 2009; 117:231–40. [PubMed: 19270793]
- Zheng H, Zeng Y, Zhang X, Chu J, Loh HH, Law PY. mu-Opioid receptor agonists differentially regulate the expression of miR-190 and NeuroD. *Mol Pharmacol*. 2010; 77:102–9. [PubMed: 19854889]
- Zhou R, Yuan P, Wang Y, Hunsberger JG, Elkahoun A, Wei Y, et al. Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. *Neuropsychopharmacology*. 2009; 34:1395–405. [PubMed: 18704095]

Outstanding questions

- What is the full repertoire of ncRNA targets and what are the neurobiological outcomes that result changes in ncRNA levels?
- Do observed changes in ncRNA expression play a causal or merely correlative role in neurotoxicological mechanisms of action?
- What are the points of entry for chemically mediated changes in ncRNA levels – changes in ncRNA transcription or stability or more broadly, in ncRNA biogenesis?
- Can paralogous ncRNAs or non-coding molecules that target different recognition elements located on the same target transcript synergize or compensate for dysregulation of ncRNA signaling following exposure to neurotoxicants?
- What role do ncRNAs play in maintaining or inhibiting feed-forward or feed-back circuitry that contributes to adverse outcomes.

Fig. 1. miRNA biogenesis. microRNAs are processed from RNA Pol II transcripts. In the canonical biogenesis pathway, ~70 nt pre-miRNA is produced by the nuclear RNase III enzyme Drosha in complex with DGCR8, a ds-RNA binding protein. Pre-miRNAs are also produced independently of Drosha/DCGR8 as a result of the splicing and debranching of short introns called mitrons. Following nuclear export, pre-miRNA molecules are bound by a second RNase III enzyme, cytoplasmic Dicer in complex with TRBP. Cleavage by Dicer yields a ~20 bp ds pri-miRNA. The miRNA duplex is separated one strand is selected as the mature or guide strand and preferentially incorporated into the AGO-containing miRISC, whereas the passenger strand is released and degraded. Under certain conditions, the passenger (star) strand is selected for miRISC incorporation. Increasingly, nomenclature modeled after the secondary structure of the pre-miRNA (5p or 3p) is used to identify the strands. The final products are incorporated into miRISC and bind to miRNA recognition elements in the 3'-UTR of target transcripts, resulting in translational repression or endonucleolytic cleavage of target mRNAs. Argonaut (AGO); double stranded (ds); miRNA-induced silencing complex (miRISC); nucleotide (nt); polymerase II (Pol II); precursor miRNA (pre-miRNA); primary miRNA (pri-miRNA); transactivation-response RNA-binding protein (TRBP).

Fig. 2.

Reprogramming miR-21 expression to influence chemotherapeutic efficacy. Glioblastoma cells express higher levels of the putative oncogene miR-21, a miRNA that functions as an anti-apoptotic factor. Overexpression of miR-21 confers partial resistance to the killing effects of the chemotherapeutic TMZ. miR-21 repression enhances the chemotherapeutic effect of taxol to human glioblastoma cells underscoring the relevance of targeting cellular miRNAs as a strategy to increase the efficacy of chemotherapeutics.

Adapted from Ren et al. (2010) and Shi et al. (2010).

Fig. 3.

Ethanol-sensitive miRNAs influence nervous system development and function and tolerance. In a fetal mouse cerebral cortex-derived culture model, ethanol exposure triggers premature neural stem-cell maturation and proliferation by repressing miR-355 expression. Ethanol also reduces levels of miR-9 and miR-153, and the anti-apoptotic miR-21 in a GABA_A-receptor dependent fashion. Functional antagonism between miR-21 and miR-335 repression overrides the pro-apoptotic effects of miR-21 repression. Repression of miR-9 and miR-153c was also reported in zebrafish embryos transiently exposed to ethanol during early neurogenesis and knockdown of miR-9 and miR-153c or exposure to ethanol results in persistent neurobehavioral effects in larval and juvenile zebrafish. Ethanol-sensitive miRNAs have also been associated with tolerance. In rat SON, ethanol exposure upregulates miR-9, a miRNA that specifically targets a subset of BK channel splice variants that contain miR-9 MREs in their 3'-UTRs. Remaining splice variants encode BK isoforms with reduced responsiveness to ethanol. Large-conductance calcium-activated and voltage-gated potassium channel (BK); miRNA recognition elements (MREs); supraoptic nucleus explants (SON).

Adapted from Pietrzykowski et al. (2008), Sathyan et al. (2007) and Tal et al. (2012).

Fig. 4.

Over-riding cocaine dependence. Rats given unrestricted access to cocaine have elevated levels of miR-212 in the dorsal striatum (Hollander et al., 2010). miR-212 represses a number of transcripts including a negative regulator of RAF1, SPRED1. SPRED1 repression allows for RAF1-mediated activation of adeny cyclase, subsequent cAMP production, and activation of the cAMP-induced CREB–TORC complex, thereby limiting cocaine intake. Activation of CREB/TORC initiates a feed-forward mechanism by upregulating miR-212 expression. miR-212 also represses MeCP2, a transcriptional repressor that promotes cocaine intake and reduces miR-212 expression. Cyclic AMP (cAMP); cAMP response element-binding (CREB); V-Raf-1 murine leukemia viral oncogene homolog 1 (RAF1); sprouty-related, EVH1 domain containing 1 (SPRED1); Transducer of regulated *CREB*-binding proteins (TORC).

Adapted from Hollander et al. (2010) and Im et al. (2010).

Fig. 5. miR-146a mediates metal neurotoxicity. Exposure to aluminum-salts promotes neuronal inflammation. In HN cells, aluminum-sulfate exposure elevates miR-146a expression. Exposure to the metal results in ROS generation and subsequent activation NF- κ B. The miR-146a gene has NF- κ B binding sites in its upstream promoter. miR-146a post-transcriptionally represses the inflammatory repressor CFH, thereby increasing neuronal inflammation. Treatment with A β 42 peptides also stimulates miR-146a-mediated repression of CFH. Aluminum sulfate ($\text{Al}_3(\text{SO}_4)_3$); amyloid beta 42 (A β 42); complement factor H (CFH).

Adapted from Pogue et al. (2009).

Fig. 6.

Proposed model of miRNA-mediated neurotoxicity. Chemical exposures alter miRNA levels by increasing or decreasing their expression, or by impacting molecule stability or turnover. Differentially expressed miRNAs post-transcriptionally repress levels of multiple target transcripts, repression of which may contribute to toxicity or adaptive responses to chemical exposures. In other instances, correlative changes in miRNA expression are not linked to observed phenotypic outcomes. miRNAs may exhibit functional synergism or redundancy at the mRNA level by targeting similar or different miRNA recognition elements on the same transcript. Post-translational repression of multiple target transcripts can synergize or antagonize toxicity or adaptive responses to neurotoxic exposures. Finally, miRNAs can participate in positive and negative feedback loops to promote or restrict further expression.

Table 1

Examples of ncRNAs.^a

Class	Name	Size (nts)	Genomic location	Function	Refs
Promoter-associated RNA	• PASR	20–200	Downstream of TSS in protein-coding genes	Unknown	Kapranov et al. (2007)
	• PROMPT	18	–2500 to –50 nts to TSS of actively transcribed protein coding genes	Transcription	Preker et al. (2008)
	• TSSa-RNA	20–90	Found within –250 to +50 nts from TSSs of highly expressed genes	Transcription	Seila et al. (2008)
	• tiRNA	18	Downstream to TSS in highly expressed genes	Transcription	Taft et al. (2009)
	• miRNA	18–24	Widespread genomic loci	mRNA cleavage or translational repression	Kim (2005)
Other small RNA	• piRNA	26–31	Intragenic	Transposon silencing, DNA methylation	Aravin et al. (2007)
	• snoRNA	60–300	Intronic	rRNA modifications	Kiss-Laszlo et al. (1996)
	• svRNA	23–40	Within vault RNA genes	Drug resistance	Persson et al. (2009)
	• TASR	20–200	3' region of genes	Unknown	Kapranov et al. (2007)
	• vRNA	88–98	Conserved genomic locus linked to protocadherin gene cluster	Transport and nuclear extrusion of xenobiotics	Gopinath et al. (2005)
Long ncRNA	• lincRNA	>1000	Intergenic	mRNA stabilization, polycomb retargeting	Mercer et al. (2009)
	• T-UCR	>350	Widespread genomic loci	Unknown	Bejerano et al. (2004)

^aComplete list of mammalian ncRNAs located at <http://research.imb.uq.edu.au/rnadb/>; long intergenic non-coding RNA (lincRNA); microRNA (miRNA); PIWI-interacting RNA (piRNA); promoter-associated small RNA (PASR); promoter upstream transcripts (PROMPTS); small nucleolar RNA (snoRNA); small vault RNA (svRNA); termini-associated small RNA (TASR); transcription start site-associated RNA (TSSa-RNA); transcription initiation RNA (tiRNA); transcribed ultraconserved region (T-UCR); vault RNA (vRNA).

Table 2

Neurotoxic and neuroactive compounds and miRNAs.

Compound	ncRNAs involved	Target genes or pathways	Function	Species or cell type	Refs
Acyclovir	↓ miR-146a	Blocks HSV-1 induced increase in miR-146a	Anti-viral defense	HN cells	Lukiw et al. (2010)
Aluminum	↑ miR-146a	Complement factor H	Pro-inflammatory	HN cells	Lukiw(2007 #159) and Pogue (2009 #172)
Aluminum/iron	miR-146a, miR-125b	?	Blocked by NFκB inhibitors	HAG cells	Pogue et al. (2011)
Cocaine	↑ miR-212	SPRED1, MeCP2	Homeostatic	Rat dorsal striatum	Hollander et al. (2010) and Im et al. (2010)
Dexamethasone	miR-29a/b	arp3	Dendritic spine remodeling	Mouse hippocampal neurons	Lippi et al. (2011)
Ethanol	↓ miR-132	BDNF-induced MAPK signaling	Blocks synaptic maturation	Mouse cortical neurons	Kumamaru et al. (2008)
	↓ miR-9, -153	?	?	Mouse cortical neuroepithelium	Sathyan et al. (2007)
	-21, -355	GABA _A -R dependent Independent of GABA _A -R	Apoptosis Cell proliferation		
	↓ miR-9, -153c	Response to light cues	Neurobehavioral development	Zebrafish	Tal et al. (2012)
	↑ miR-9	Subset of BK channel splice variants	Tolerance	Rat SON explants	Pietrzykowski et al. (2008)
Fluoxetine	↑ miR-16	Blocks SERT translation	Anti-depressant	Serotonergic cells and raphe nuclei	Baudry et al. (2010)
Lithium/valproate	↓ miR-34a	GRM7?	Anti-psychotic	Rat hippocampal neurons	Zhou et al. (2009)
Morphine	↓ miR-133b	pitx3	Differentiation	Zebrafish and rat hippocampal neurons	Sanchez-Simon et al. (2010)
Nicotine	↑ miR-140*	dnm1	Synaptic function	PC12 cells	Xu and Li (2011)
	↓ miR-29a/b	arp3	Dendritic spine remodeling	Mouse hippocampal neurons	Lippi et al. (2011)
RDX	↑ miR-206, -30a-5p, -195	BDNF?	Repress BDNF signaling	Mice	Zhang and Pan (2009)
Temozolamide	miR-21	Overexpression increases Bax/Bcl2 ratio	Drug resistance	Human glioma cells	Shi et al. (2010)
Taxol	miR-21	Knockdown decreases Bax/Bcl2 ratio	Increased drug efficacy	Human glioma cells	Ren et al. (2010)

BCL2-Associated X Protein (Bax); B-cell lymphoma 2 (Bcl2); large-conductance calcium-activated and voltage-gated potassium channel (BK); Brain Derived Neurogenic Factor (BDNF); dynamin 1 (dnm1); Gamma-Aminobutyric Acid Receptor α subunit (GABAA-R); metabotropic glutamate receptor 7 (GRM7); Human Astroglial cells (HAG cells); Herpes Simplex Virus 1 (HSV-1); Human Neural cells (HN cells); Mitogen Activated Protein Kinase (MAPK); methyl CpG binding protein 2 (MeCP2); pituitary homeobox 3 (pitx3); serotonin transporter (SERT); supraoptic nucleus (SON); sprouty-related, EVH1 domain containing 1 (SPRED1).