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Emerging roles for ncRNAs in alcohol use disorders

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Abstract

Chronic alcohol exposure produces widespread neuroadaptations and alterations in gene expression in human alcoholics and animal models. Technological advances in the past decade have increasingly highlighted the role of non-protein-coding RNAs (ncRNAs) in the regulation of gene expression and function. These recently characterized molecules were discovered to mediate diverse processes in the central nervous system, from normal development and physiology to regulation of disease, including alcoholism and other psychiatric disorders. This review will investigate the recent studies in human alcoholics and rodent models that have profiled different classes of ncRNAs and their dynamic alcohol-dependent regulation in brain.

Keywords

alcohol; non-coding RNA; microRNA; long noncoding RNA; transcriptome; gene regulation; next generation sequencing

II. Introduction

Non-coding RNA

Protein-coding genes have traditionally been the most well studied sequences in the human genome; however, these genes account for less than 2% of known structural and regulatory molecular elements (Alexander, Fang, Rozowsky, Snyder, & Gerstein, 2010). In recent years, it has become increasingly clear that the non-protein-coding portion of the genome is functionally important and is required for normal development and physiology, and is also linked with a number of diseases (Cech & Steitz, 2014; Mercer, Dinger, & Mattick, 2009). This fundamental change in our understanding of the complexity of the transcriptome has been the result of improved RNA sequencing (RNA-seq) technologies, which allow exploration of gene structure and regulation in unprecedented detail (Fig. 1). Data generated from these advanced techniques have transformed our view of the central dogma that DNA is transcribed into RNA, which is translated into protein. Non-coding RNAs (ncRNAs) are

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emerging as key transcriptional and post-translational regulators, representing a large and diverse class of regulatory molecules (Alexander et al., 2010). ncRNAs make up a sizeable portion of the transcriptional landscape of the cell (Carninci & Hayashizaki, 2007; Carninci et al., 2005), but the precise functions of many non-coding elements remain largely unknown. Defining the biological roles carried out by multiple classes of ncRNAs is an expanding area of transcriptomics that will likely rival the large number and diversity represented by the proteome. Currently, ncRNAs are grouped into three general subclasses based on nucleotide number (small, 18–31 nt; medium, 31–200 nt; and long, >200 nt), with each class having regulatory potential and specific subcellular localization (Alexander et al., 2010; Costa, 2005; Dozmorov, Giles, Koelsch, & Wren, 2013). These ncRNAs include transfer and ribosomal RNA (tRNA and rRNA, respectively), small nucleolar RNA (snoRNA), microRNA (miRNA), small interfering RNA (siRNA), small nuclear (snRNA), extracellular RNA (exRNA), piRNAs, and small Cajal body-specific RNA (scaRNA), as well as different classes of long ncRNA (lncRNA), including intergenic (lincRNA) and intronic RNA. Identifying roles for ncRNAs will provide a better understanding of cellular function, as well as new insight into gene regulation of disease.

Genomic studies based on DNA and RNA sequencing have identified thousands of ncRNAs in diverse animal genomes. Until recently, the conservation of ncRNAs between human and nonhuman primates was limited compared to human-mouse conservation, due to limitations in the quality of nonhuman primate genome annotations. However, sequencing technology has resulted in more complete and correct assemblies, thus greatly improving genome annotation quality. Systematic curation efforts have enabled the development of several cross-species ncRNA databases (Ulitsky, 2016). Identification of cross-species conservation is a key question when evaluating the functional impact of specific ncRNAs. For example, if a ncRNA is associated with a human condition, it is important to know the extent to which it can be studied in model organisms. Conversely, if a ncRNA is discovered in a model organism, evidence of conservation will be critical for establishing relevance to a human condition.

Distinct ncRNA mechanisms in brain are thought to influence the development of psychiatric diseases (Kocerha, Dwivedi, & Brennand, 2015; Sartor, St Laurent, & Wahlestedt, 2012), including alcoholism (Farris & Mayfield, 2014). Rapid advances in this field have largely come from next-generation sequencing technologies such as RNA-seq that have been critical for genome-wide identification of novel transcripts (Fig. 1). Previous review articles have focused on ncRNAs (primarily miRNAs) that are altered in response to alcohol administration (Balaraman, Tingling, Tsai, & Miranda, 2013; Farris & Mayfield, 2014; Most, Workman, & Harris, 2014; Nunez & Mayfield, 2012; Pietrzykowski, 2010). This review focuses on the effects of alcohol on ncRNAs from studies published since 2012. It should be noted that there are few studies focusing on the association of genetic risk to ncRNAs in animal models, representing an area of future research.

III. MicroRNA

The expanding fields of genetics and genomics over the previous 15 years have highlighted the growing number of genes that can potentially influence alcohol-drinking behavior in

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humans and animal models. In particular, the discovery of miRNAs (Lee, Feinbaum, & Ambros, 1993) and their mechanisms of action are revolutionizing our understanding of gene regulation in physiology and disease (Mattick & Makunin, 2006; Morris & Mattick, 2014). These short (~17–24 nt) ncRNAs act as post-transcriptional modulators of gene expression by binding to miRNA-recognition elements in their numerous target genes. miRNA-mediated gene suppression occurs through multiple mechanisms, including interruption of translational initiation, 5′ decapping, alternative splicing, 3′ deadenylation, and exonuclease degradation (Fabian & Sonenberg, 2012; Krol, Loedige, & Filipowicz, 2010). miRNA biogenesis includes gene transcription by RNA polymerase II that typically binds to promoters near DNA sequences encoding precursor miRNAs (pre-miRNA). The resulting transcript is capped, polyadenylated, and spliced (Hammond, 2015). Many pre-miRNAs are derived from intronic transcriptional regions; however, novel mechanisms have been identified demonstrating that traditional RNA splicing events can negatively regulate the processing of pre-miRNAs that overlap exon-intron junctions (Melamed et al., 2013). These distinctly different RNA processing mechanisms (miRNA processing and RNA splicing) underscore the regulatory potential of these short ncRNAs.

miRNAs play important roles in neuronal differentiation, developmental timing, synapse function, and neurogenesis (Fiorenza & Barco, 2016). miRNAs are thought to act as ‘master regulators’ of gene expression, and a recent RNA-seq study of 13 different types of human tissue identified over 3,700 mature miRNAs (Londin et al., 2015), compared to the >2,700 listed in release 20 of miRBase (Kozomara & Griffiths-Jones, 2014). In addition, the sequence conservation across human and nonhuman primate-specific lineage is quite high (>94% of the newly discovered miRNAs). Given the vast number of miRNAs identified to date and the expression silencing of large collections of target genes, there is considerable regulatory potential of these molecules.

Alcohol-responsive miRNAs in human postmortem brain

Because of their regulatory functions, it is reasonable to expect that miRNAs are also critical mediators of alcohol’s effects. Early studies demonstrated that alcohol alters miRNA levels and miRNA-regulated systems that are associated with tolerance, gut leakiness, and neural stem cell proliferation and differentiation (Miranda et al., 2010; Pietrzykowski et al., 2008). Similarly, expression-profiling studies in postmortem brains of human alcoholics have shown that the transcriptional reprogramming that takes place is brain region-specific and may reflect both pre-existing differences in gene expression and alterations in response to alcohol consumption (Nunez & Mayfield, 2012; Nunez, Truitt, Gorini, Ponomareva, Blednov, et al., 2013). In addition, epigenetic reprogramming primarily mediated by direct methylation of DNA and acetylation, methylation, and phosphorylation of histone proteins, appears to contribute to the altered gene expression observed in alcoholics and animal models of excessive alcohol consumption (Krishnan, Sakharkar, Teppen, Berkel, & Pandey, 2014; Miranda, 2014). The first transcriptome-wide study of alcohol-responsive miRNAs in human alcoholics identified ~35 upregulated human miRNAs in the prefrontal cortex (PFC) (Lewohl et al., 2011). This study included both miRNA and mRNA whole genome microarrays and used integrative statistical analyses to demonstrate that the predicted mRNAs targeted by upregulated miRNAs were significantly over-represented among the

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downregulated mRNAs, with no over-representation detected among the set of significantly upregulated mRNAs. This supports a role for miRNA-dependent inhibition of gene expression in the PFC of human alcoholics. The magnitude of changes in miRNA levels was relatively small (20–30%), with few changes exceeding 40%. It is likely that the small changes detected in miRNAs, as well as mRNA expression, in PFC of human alcoholics is due to an increased expression that is localized to a specific cellular compartment, such as the neuronal synapse. Such compartmentalized, enhanced differential expression has been demonstrated in an animal model where miRNAs were isolated from synaptoneurosomes when compared to total, unfractionated tissue (Most, Leiter, Blednov, Harris, & Mayfield, 2016).

Another whole genome study examined miRNA expression from frontal cortex (Broadman area 9) of human alcoholics to further characterize the effects of alcohol consumption on predicted target mRNA expression (Manzardo, Gunewardena, & Butler, 2013). Similar to Lewohl et al. (2011), the majority of differentially expressed miRNAs were upregulated in alcohol-dependent subjects compared with controls, including a cluster of four miRNAs (miR-299-3p, miR-377, miR-379, and miR-493) from the maternally expressed 14q32 chromosomal region. The predicted mRNA targets of these upregulated miRNAs were involved in cellular adhesion, tissue differentiation, neuronal migration, myelination, and oligodendrocyte proliferation. These findings suggest that white matter abnormalities observed in alcoholism may be linked to upregulated miRNAs from this chromosomal region, and are consistent with the earlier finding that upregulation of miRNAs in the PFC of alcoholics targets mRNAs important for lipid biosynthesis and myelination (Lewohl et al., 2011).

The whole transcriptome studies outlined above profiled miRNA levels from total homogenate preparations, but we must consider that miRNA regulation of gene expression may specifically shape synaptic structure and function. Synaptoneurosomes (SNs) contain membrane vesicles of pre- and postsynaptic compartments of neurons and perisynaptic compartments of astrocytes and microglia (Raab-Graham, Haddick, Jan, & Jan, 2006), and provide a model for studying the synaptic transcriptome. Alcohol-responsive miRNAs have been examined in SNs prepared from amygdala of mice following chronic two-bottle choice (2BC) drinking (Most et al., 2016). This research was microarray-based and used different informatics approaches to identify key alcohol-sensitive miRNA-mRNA synaptic interactions. These miRNAs and mRNAs demonstrated overlapping patterns of expression that correlated with alcohol consumption, and a significant number of the alcohol-responsive mRNAs and miRNAs were unique to glutamate neurons. These findings point to the utility of the SN preparation in studying cell-specific signaling and the ability of chronic alcohol to perturb coordinated miRNA regulation of mRNAs as a mechanism to disrupt synaptic plasticity and thereby alter brain function. Some of the alcohol-sensitive synaptic miRNAs in mouse brain were also found in human (miR-18a, miR-203, miR-369*, miR-92a, and miR-423) (Lewohl et al., 2011) and rat brain (miR-137, miR-187, miR-18a, miR-34c*, miR-369*, miR-374, miR-382*, miR-423, miR-488, and miR-92b) (Tapocik et al., 2013), suggesting that overlapping alcohol-responsive miRNAs are conserved across different species and models of alcohol consumption.

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Recently, bioinformatic approaches were used to overlay gene networks from both miRNAs and mRNAs in the nucleus accumbens (NAc) of postmortem brain (Mamdani et al., 2015). Weighted gene co-expression network analysis (WGCNA; Langfelder & Horvath, 2008) was used to define networks of co-expressed genes from genome-wide miRNA and mRNA datasets to identify multiple coding- and ncRNA gene modules that correlated significantly with DSM-IV alcohol dependence. This study included an additional layer of analyses that combined network hub gene expression with genome-wide genotypic data from an independent genetic sample from the Collaborative Studies on the Genetics of Alcoholism (COGA) to identify numerous mRNA and miRNA cis-eQTLs significantly enriched for alcohol dependence, representing an important step toward identification of alcohol-relevant eQTLs from gene expression in human postmortem brain. This study also identified cell-type specific mRNA modules that were enriched for both neuronal genes (primarily downregulated), as well as astrocyte and microglial marker genes (primarily upregulated). Among these modules, multiple functional categories of genes were enriched with MAPK and cytokine signaling pathways, consistent with early studies that identified genes involved in neuroimmune signaling and function from human alcoholics and rodent models of excessive drinking (Liu et al., 2006; Mayfield, Ferguson, & Harris, 2013; Mulligan et al., 2006; Robinson et al., 2014).

Alcohol-responsive miRNAs in rodent models

Rodent drinking models have been instrumental in defining alcohol-responsive miRNAs and their functional relevance based on responses to expression manipulation. These animal models are advantageous because select miRNAs can be upregulated or downregulated using viral vectors, mimics (molecules designed to simulate naturally occurring mature miRNAs), and antagomirs (artificial antisense oligonucleotides) to determine their effect on alcohol-related behaviors. Studies have demonstrated that alcohol-induced changes in miRNAs are associated with cellular tolerance to alcohol (Pietrzykowski et al., 2008), antianxiety effects (Teppen, Krishnan, Zhang, Sakharkar, & Pandey, 2015), cellular reward mechanisms (Li et al., 2013), regulation of alcohol consumption and preference (Bahi & Dreyer, 2013; Li et al., 2013; Most et al., 2016; Tapocik et al., 2014), episodes of binge drinking (Darcq et al., 2015; Nunez, Truitt, Gorini, Ponomarev, Harris, et al., 2013; Tian et al., 2016), dependence/withdrawal (Gorini, Nunez, & Mayfield, 2013; Tapocik et al., 2013, 2014), and alcohol-induced conditioned-place preference (Bahi & Dreyer, 2013). Recent research articles published since 2012 are listed in Table 1.

A comprehensive and integrative analysis of alcohol-responsive miRNAs (Exiqon miRNA arrays) and proteins (2-dimensional differential in-gel electrophoresis followed by MALDI tandem mass spectrometry) from mouse cortex and midbrain was reported using a mouse model of voluntary alcohol consumption and dependence (Gorini, Nunez, et al., 2013; Gorini, Roberts, & Mayfield, 2013). Bioinformatic analyses identified modules of co-expressed miRNAs highly correlated with predicted target genes encoding differentially expressed proteins (Gorini, Roberts, et al., 2013). Overall, miRNA profiles from alcohol-dependent animals segregated into clear clusters of predominantly upregulated miRNAs that correlated with predominantly downregulated proteins. Key regulatory molecules were associated with the escalation of alcohol consumption to dependence (e.g., miR-532-3p and

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miR-339-5p on Pea15 in the midbrain), and included genes encoding co-expressed proteins that are targeted by the same miRNA (e.g., miR-494-3p on both Dpysl2 and Dpysl3 and miR-140-3p on co-expressed Flot1 and Dnm1 in the cortex). This analysis of global miRNA and protein expression levels from different brain regions of alcohol-dependent mice suggests a synergistic regulation of miRNAs and proteins in the behavioral transition from alcohol consumption to dependence.

A separate study utilizing a rat model of alcohol dependence examined the relationships among miRNAs and mRNAs that are correlated with genes involved in disruption of synaptic processes and neuroplasticity (Tapocik et al., 2013). Multiple miRNAs, a number of which are also differentially expressed in human alcoholics (Lewohl et al., 2011), and mRNAs were differentially expressed in the medial PFC (mPFC) in response to chronic alcohol exposure in rats. This study identified miR-206, a primarily upregulated miRNA target which has also been implicated to have functional relevance with brain-derived neurotrophic factor (BDNF) expression (Duman & Monteggia, 2006; Hansson, Rimondini, Heilig, Mathé, & Sommer, 2011). BDNF, an activity-regulated neurotrophin with known 3′-UTR binding sites for miR-206, was a confirmed downregulated mRNA. Building upon these findings, subsequent research confirmed that miR-206 is upregulated in a brain region-specific manner in response to dependence-induced drinking. Upregulation was observed only in mPFC and not in the ventral tegmental area, amygdala, or NAc (Tapocik et al., 2014). Viral-mediated overexpression of miR-206 decreased BDNF protein expression and induced escalation of alcohol self-administration in nondependent rats, further supporting a role for miR-206 in alcohol dependence. This study provides strong evidence for a direct role of miR-206 in escalation of alcohol self-administration.

Rodent studies designed to model binge-like drinking in humans have identified numerous alcohol-responsive miRNAs. For example, whole-genome miRNA studies found many differentially regulated miRNAs in binge-alcohol models in adolescent rats (Prins, Przybycien-Szymanska, Rao, & Pak, 2014; Tian et al., 2016) and adult mice (Gorini, Nunez, et al., 2013; Most et al., 2016; Nunez, Truitt, Gorini, Ponomarev, Harris, et al., 2013). Using a 2BC-drinking in the dark (DID) paradigm in mice bred for high alcohol consumption (C57BL/6J × FVB/NJ hybrid), Nunez and colleagues (Nunez, Truitt, Gorini, Ponomarev, Harris, et al., 2013) found that miRNA expression was predominantly upregulated in the PFC of alcohol-drinking mice (52 miRNA families), a finding consistent with previous results from human alcoholic PFC (Lewohl et al., 2011). In addition, approximately 40–80% of the common differentially expressed genes were changed in opposite directions in human and mouse models, with the majority of genes being upregulated in the alcohol-treated mouse brain but downregulated in human alcoholic brain. These results demonstrate 1) a high degree of conservation of alcohol-responsive genes and pathways from mouse to human and 2) unique differences in the direction of gene expression changes between the two models. These distinctions may be a consequence of the duration of alcohol exposure, which lasted for most of the adult life in the case of the human alcoholics (long-term adaptations), but only 20 days during early adulthood in the binge-drinking mice (early stages of adaptation). Co-expression network analysis identified modules of genes affected by alcohol and miRNAs with biological functions that include synaptic vesicle-mediated transport, endocytic recycling, and neuroimmune signaling mediated by chemokine and

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Toll-like receptor signaling. The pathways that were both upregulated by alcohol and over-targeted by upregulated miRNAs were enriched in downregulated modules, suggesting that these may be involved in the early stages of chronic alcohol consumption and trigger a miRNA adaptive response aimed at counteracting such activation. miR-34c-5p and let-7g-5p, in particular, appear to be central regulators of hub genes in the alcohol-responsive gene modules. Differentially expressed and PCR-validated miRNA families that were identified in both mouse and human included let-7, miR-7, miR-15, miR-101, miR-140, and miR-152, and the validated miRNAs specific to the mouse model included miR-195 (member of the miR-15 family) and miR-541 family members. There was also significant overlap among differentially expressed mouse miRNA families and those reported in PFC of ethanol-treated rats (Tapocik et al., 2013). The miRNA families with altered expression in both mice and rats included miR-7, miR-9, miR-10, miR-15, miR-17, miR-26, miR-29, miR-30, miR-101, miR130, miR-181, miR-204, miR-339, miR-340, miR-368, miR-434, and miR-467. Overall, these results underscore the relevance of gene regulation by miRNAs in response to alcohol consumption and suggest conservation of alcohol-responsive miRNA regulatory pathways from rodent to human.

In contrast to the effects of alcohol on BDNF signaling pathways outlined above (Tapocik et al., 2013, 2014), BDNF mRNA increased in the dorsal striatum of rodents following moderate levels of alcohol intake in a chronic continuous-access test (Bahi & Dreyer, 2013) and an operant model of self-administration (Jeanblanc et al., 2009), and the increase was attenuated by prolonged daily exposure to alcohol (Jeanblanc et al., 2009). Prolonged access also resulted in decreased BDNF levels in the cortex, suggesting that binge alcohol consumption dysregulates BDNF expression in corticostriatal brain pathways (Jeanblanc et al., 2009). A subsequent study by the same group found that BDNF mRNA levels were also decreased in mPFC using a model that mimics binge alcohol drinking in humans (Darcq et al., 2015), and these changes were accompanied by increased expression levels of miR-3-a-5p, miR-195 and miR-1. A similar increase in miR-1 was seen in the PFC of human alcoholics (Lewohl et al., 2011). Detailed follow-up studies indicated that overexpression of miR-30a-5p (using viral vectors) in the mPFC decreased BDNF expression and increased alcohol intake and preference, whereas knockdown (LNA-miRNA; antagomers) decreased excessive alcohol drinking (Darcq et al., 2015). These findings, as well as those from adolescent binge models (Prins et al., 2014), suggest that there is a dynamic miRNA-dependent regulation of the BDNF pathway that is associated with the transition from moderate to uncontrolled alcohol intake.

IV. Long Non-coding RNA

Long non-coding RNAs (lncRNAs; >200 nt) are the most abundant class of RNAs. Compared to the approximately 20,000 protein-coding genes in humans (Carninci & Hayashizaki, 2007; Jia et al., 2010; Ravasi, 2006), current annotations indicate that there are >16,000 lncRNAs, the majority of which show tissue- and temporal-specific expression in the CNS (Derrien et al., 2012). lncRNAs are critical to normal cellular function, including the regulation of gene expression (Wang & Chang, 2011), cell proliferation and differentiation (Guttman et al., 2011), as well as the pathophysiology of disease (Wapinski & Chang, 2011). The expression level of lncRNAs is highest in brain (Derrien et al., 2012), and

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similar to protein-coding genes; they are regulated by neuronal activity (Barry et al., 2014) and show cell type- and brain region-specific expression patterns (Belgard et al., 2011; Mercer, Dinger, Sunkin, Mehler, & Mattick, 2008), suggesting that their expression is important in discrete CNS functions. Although the physiological roles of lncRNAs are still emerging, they appear to be involved in cis-regulation of neighboring genes (Goff et al., 2015; Zuo et al., 2016) and the regulation of gene expression involved in adaptive behavior (Bekdash & Harrison, 2015; Spadaro et al., 2015). The extent to which specific lncRNAs contribute to the development and maintenance of alcohol- or other substance-abuse disorders is currently an under-investigated area, with the potential to uncover novel regulatory gene networks involved in the addictive process.

According to the positional relationship between lncRNAs and their associated protein-coding genes, lncRNAs are classified as intergenic (lincRNA; located between protein-coding genes and at least 1 kb away from the nearest protein-coding genes), intronic (located within the intron of annotated protein-coding genes), anti-sense (RNA molecules that are transcribed from the opposite strand of many protein-coding genes), and sense overlapping (considered transcript isoforms of protein-coding mRNAs) (Zuo et al., 2016).

lncRNA and psychiatric disease

Data from psychiatric and substance-abuse disorders (Sartor et al., 2012) clearly demonstrate regulatory roles for these molecules; however, there are few studies that establish a direct causal link between lncRNAs and regulation of drinking in animal models or humans. As data rapidly accumulate, it will remain a challenge to determine which lncRNAs are associated with specific disease states and to identify the mechanisms and regulatory pathways that underlie these conditions. Current approaches such as sequence classification, association studies, differential expression, and informatic analyses have been useful in predicting the regulatory potential of lncRNAs in psychiatric conditions, including Alzheimer's disease (Faghihi et al., 2008; Magistri, Velmeshev, Makhmutova, & Faghihi, 2015), schizophrenia (Barry et al., 2014; Perkins, Jeffries, & Sullivan, 2005), autism spectrum disorders (Ziats & Rennert, 2013), as well as substance dependence (Sartor et al., 2012; Sullivan, Daly, & O'Donovan, 2012; Zuo et al., 2016) discussed in the following section.

lncRNA and alcohol dependence

To date, there have been few studies of how lncRNAs are regulated by alcohol exposure, but alterations in lncRNAs have been reported following nicotine, heroin, and cocaine exposure (Sartor et al., 2012; Zuo et al., 2016). The majority of alcohol research has focused on miRNAs; however, there is accumulating evidence that lncRNAs are also involved in synaptic plasticity changes associated with drug abuse and dependence. These transcripts are known to mediate epigenetic factors that affect gene expression (Khalil et al., 2009; Lee, 2012; Wang et al., 2011), act as endogenous competitors (Cesana et al., 2011), regulate alternative splicing events (Barry et al., 2014; Massone et al., 2011; Tripathi et al., 2010), control neuronal development (Pollard et al., 2006), and guide synaptic plasticity (Bond et al., 2009). These diverse roles suggest that lncRNA expression may prove to have significant regulatory control in alcohol dependence and other psychiatric diseases.

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Transcriptome studies

There are few studies of lncRNA dysregulation in alcohol dependence, and none that identify mechanisms underlying functional changes in response to chronic alcohol use. Interestingly, there may be overlap in drug-responsive lncRNAs altered by alcohol dependence and other substance-abuse disorders. For example, nuclear-enriched abundant transcript 2 (NEAT2), an lncRNA important in synaptogenesis (Bernard et al., 2010), was upregulated in human alcoholic brain (Kryger, Fan, Wilce, & Jaquet, 2012). NEAT2, and other related lncRNAs, were also upregulated in the NAc of heroin (e.g., NEAT1, MIAT, and MEG3) and cocaine abusers (e.g., MIAT, MEG3, and EMX2OS) (Albertson et al., 2004; Michelhaugh et al., 2011). Thus, generalized substance abuse/dependence may produce overlapping changes in related lncRNAs.

Transcriptional network analyses using RNA-seq have also been used to identify networks of co-expressed genes that include lincRNAs such as LINC00092, LINC00174, and LINC00284 (Farris, Arasappan, Hunicke-Smith, Harris, & Mayfield, 2014). The function of these ncRNAs is currently unknown, but may involve gene regulation in human disease (Mercer et al., 2009). The co-expression patterns of these lincRNAs suggest a cooperative role in cellular plasticity that shapes patterns of human alcohol consumption. RNA-seq analysis of postmortem alcoholic brain tissue (Farris & Mayfield, unpublished) identified over 130 differentially expressed intergenic lncRNAs ($p < 0.05$) across four brain regions. The RNA-seq analysis was based on conservative estimates of gene expression according to the RefSeq database, which focuses on curated genome sequences.

Epigenetic studies (methylation)

Epigenetic studies have established a link between alcohol use and hypomethylation of genomic regions critical for embryonic development (Ouko et al., 2009). There was a pattern of increased demethylation at H19, a lncRNA known to regulate cell proliferation and imprinting, that correlated with alcohol consumption (Gabory et al., 2009), demonstrating an association between chronic alcohol use and demethylation of normally hypermethylated regions of DNA.

Genome-wide association studies

A survey of genome-wide association studies (GWAS) of alcohol dependence identified potential biological functions for the reported risk variants (Zuo et al., 2014). For example, variants located within an alcohol dehydrogenase (ADH) cluster included a large antisense-overlapping lncRNA, LOC100507053 (Xu et al., 2015), covering multiple loci for *ADH* genes that form a risk-genomic region for alcohol dependence identified by GWAS and candidate gene studies (Gelernter et al., 2009; Li, Zhao, & Gelernter, 2011; Xu et al., 2015). This class of antisense-overlapping lncRNAs frequently uses diverse transcriptional and post-transcriptional regulatory mechanisms to fulfill a wide variety of biological roles (Sartor et al., 2012; St Laurent, Wahlestedt, & Kapranov, 2015), and thus may significantly regulate cellular responses to alcohol.

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V. Conclusions

Recent technological advances in genetics and genomics have dramatically changed our view of cellular regulation. RNA-seq and other technologies have identified several classes of ncRNAs and expanded a new area of transcriptomics that is not only revolutionizing our understanding of the diversity of the proteome and cellular regulatory mechanisms, but also our insight into the genetic regulation of disease. Because ncRNAs have proven to be important for normal development and synaptic physiology, it follows that they are also key regulators in pathological conditions such as alcoholism (Fig. 2). Studies have identified alcohol-sensitive miRNAs and lincRNAs that are involved in the transition from excessive alcohol consumption to dependence, potentially silencing large collections of target genes. The regulation of gene expression by ncRNAs plays an important role in adaptive behavior, but the extent to which these molecules contribute to the development and maintenance of alcohol dependence or other substance-abuse disorders remains largely unknown. A better understanding of mechanisms involved in alcohol-mediated ncRNA changes could advance therapeutic strategies for this disease. This review has outlined numerous ncRNAs that respond to various challenges by alcohol, and examples are outlined above demonstrating that ncRNA manipulation can alter drinking behavior. Clearly, additional work is required to move this type of basic research to a clinical setting. There has been a tremendous effort to develop synthetic or expressed ncRNA mimics to treat other disease conditions, particularly cancer (Matsui & Corey, 2016; Roberts & Wood, 2013), and a number of biotechnology companies have focused on developing oligonucleotide-based drug chemistries for use in clinical trials (Matsui & Corey, 2016). This novel use of synthetic oligonucleotides to influence ncRNA and ncRNA activity is an exciting prospect for future AUD therapies.

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Highlights

- Technological advances in the previous decade have increasingly highlighted the role of non-protein-coding RNAs (ncRNAs) in the regulation of gene expression and function.
- These recently characterized molecules were discovered to mediate diverse processes in the central nervous system, from normal development and physiology to regulation of disease, including alcoholism and other psychiatric disorders.

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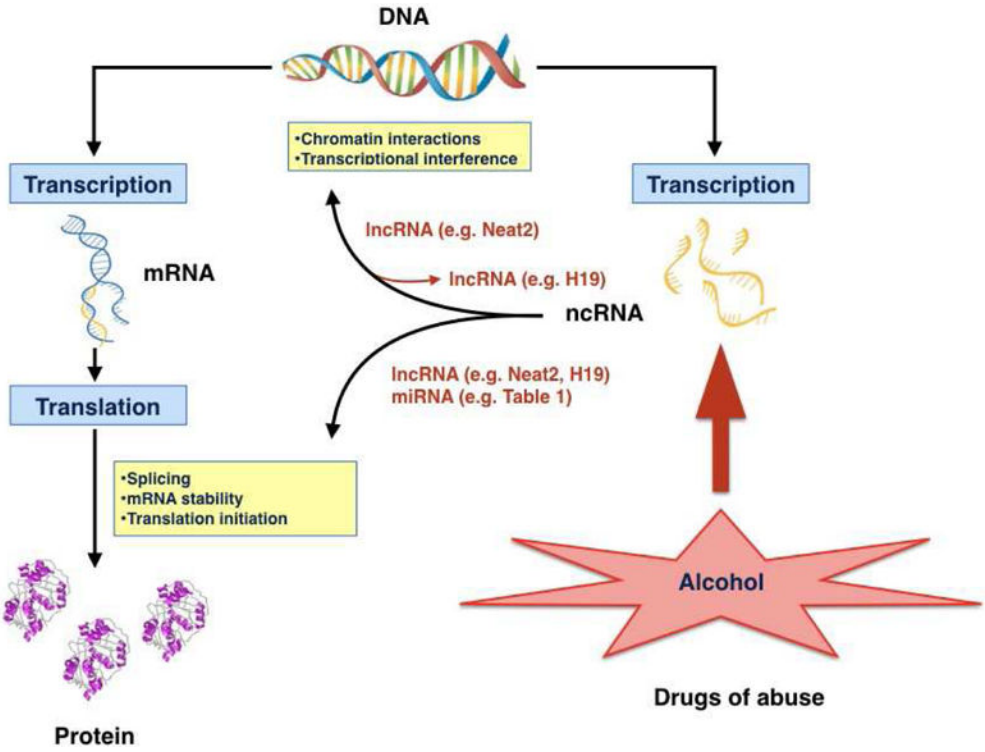

Fig. 1.

RNA features detected by RNA-seq in the prefrontal cortex of alcoholic and matched control samples. Bar plots depict the percentage of features detected in representative control (blue) and alcoholic (red) samples. The left axis shows the percentage of features for the top three biotypes and the right axis shows the percentage of remaining biotypes (separated by dotted green vertical line). Protein-coding transcripts were the predominant feature detected in both groups.

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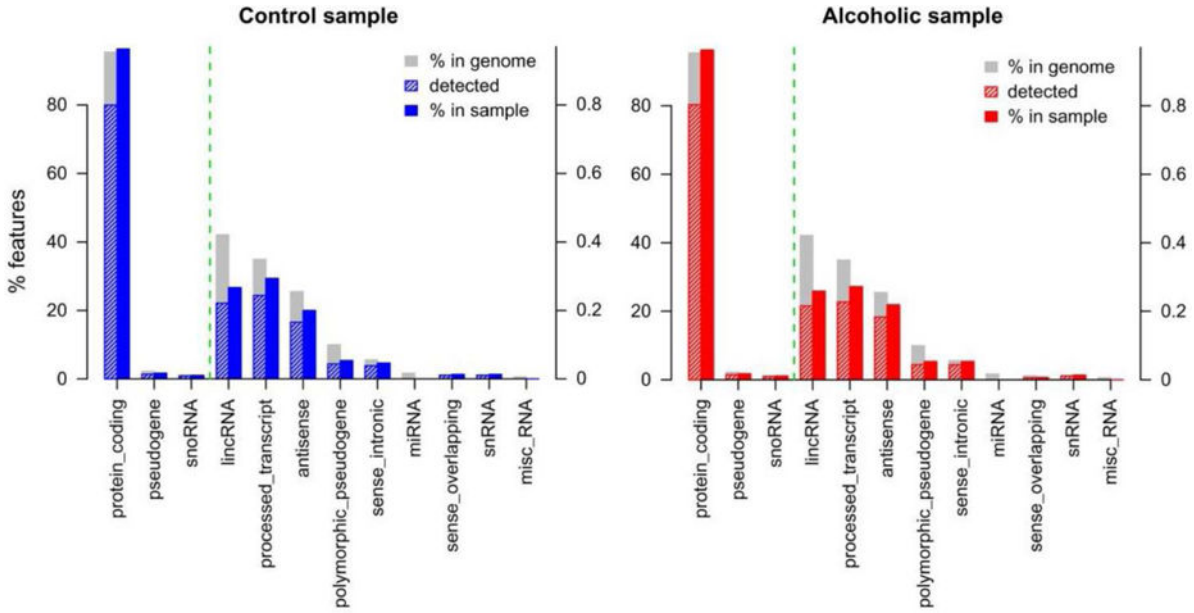

Fig. 2.

Cartoon summary of alcohol-mediated regulation of ncRNAs. The blue and yellow boxes represent the “central dogma” concept of gene expression at the transcriptional or translational levels, and the potential mechanisms for ncRNA regulation are illustrated in red. Examples of lncRNA-mediated regulation of epigenetic factors (Neat2) and alcohol-induced changes in lncRNA methylation (H19) are shown. Alcohol may regulate both lncRNAs and miRNAs at the protein translational level.

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Table 1

MicroRNAs implicated in alcohol studies (2012 to 2016).

Species/Model	Region/System	miRNA(s)	Citation(s)
Human	*PFC	miR-130a ↓	Wang, Gelernter, & Zhang, 2013
	*FC (Broadmann 9)	multiple ↑	Manzardo et al., 2013
	*NAc	multiple ↑↓	Mamdani et al., 2015
Rat	*DLS	miR-124a ↓	Bahi & Dreyer, 2013
	*NAc	miR-382 ↓	Li et al., 2013
	*mPFC	multiple ↑↓	Tapocik et al., 2013
	Hippocampus	miR-10a-5p, miR-26a, miR-103, miR-495	Prins, Przybycien-Szymanska, Rao, & Pak, 2014
	*mPFC	miR-206 ↑ manipulation[a]	Tapocik et al., 2014
	Amygdala	miR-494 ↓	Teppen et al., 2015
	*PFC	miR-125a-5p ↓	Tian et al., 2016
Mouse	*FC and midbrain	multiple ↑↓	Gorini, Nunez, & Mayfield, 2013
	*FC	multiple ↑↓	Nunez, Truitt, Gorini, Ponomareva, Harris, et al., 2013
	*mPFC	miR-30a-5p ↑ manipulation[a]	Darcq et al., 2015
	Amygdala	multiple ↑↓	Most et al., 2016
Cell Culture	Primary mouse cortical neurons	miR-155, miR-186, miR-24, and miR-375 ↑	Bekdash & Harrison, 2015
	Primary mouse microglial cells	miR-339-5p ↑	Zhang, Wei, Di, & Zhao, 2014
	Primary rat cortical neurons	miR-125a-5p ↓ manipulation[a]	Tian et al., 2016

*Frontal cortex (FC); Prefrontal cortex (PFC); Medial prefrontal cortex (mPFC); nucleus accumbens (NAc); dorsal striatum (DLS)

↑upregulated; ↓downregulated; ↑↓both up- and down-regulation of multiple miRNAs

[a]manipulation; specific manipulation with viral vectors, miRNA mimics, or antagomirs