Gene 526 (2013) 356–363

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Over-expression of the miRNA cluster at chromosome 14q32 in the alcoholic brain correlates with suppression of predicted target mRNA required for oligodendrocyte proliferation ☆

A.M. Manzardo [a,*], S. Gunewardena [c,d], M.G. Butler [a,b]

[a] Department of Psychiatry and Behavioral Sciences, University of Kansas School of Medicine, Kansas City, KS, USA
[b] Department of Pediatrics, University of Kansas School of Medicine, Kansas City, KS, USA
[c] Department of Molecular and Integrative Physiology, University of Kansas School of Medicine, Kansas City, KS, USA
[d] Department of Biostatistics, University of Kansas School of Medicine, Kansas City, KS, USA

ARTICLE INFO

Article history:
Accepted 23 May 2013
Available online 6 June 2013

Keywords:
miRNA expression
Exon expression
Alcoholism
Human frontal cortex
14q32 chromosome region
Oligodendrocyte proliferation

ABSTRACT

We examined miRNA expression from RNA isolated from the frontal cortex (Broadman area 9) of 9 alcoholics (6 males, 3 females, mean age 48 years) and 9 matched controls using both the Affymetrix GeneChip miRNA 2.0 and Human Exon 1.0 ST Arrays to further characterize genetic influences in alcoholism and the effects of alcohol consumption on predicted target mRNA expression. A total of 12 human miRNAs were significantly up-regulated in alcohol dependent subjects (fold change ≥ 1.5, false discovery rate (FDR) ≤ 0.3; $p < 0.05$) compared with controls including a cluster of 4 miRNAs (e.g., miR-377, miR-379) from the maternally expressed 14q32 chromosome region. The status of the up-regulated miRNAs was supported using the high-throughput method of exon microarrays showing decreased predicted mRNA gene target expression as anticipated from the same RNA aliquot. Predicted mRNA targets were involved in cellular adhesion (e.g., *THBS2*), tissue differentiation (e.g., *CHN2*), neuronal migration (e.g., *NDE1*), myelination (e.g., *UGT8*, *CNP*) and oligodendrocyte proliferation (e.g., *ENPP2*, *SEMA4D1*). Our data support an association of alcoholism with up-regulation of a cluster of miRNAs located in the genomic imprinted domain on chromosome 14q32 with their predicted gene targets involved with oligodendrocyte growth, differentiation and signaling.

1. Introduction

RNA has emerged as a major component of the regulatory circuitry in cells of complex organisms including humans. Less than 2% of the mammalian genome encodes protein, thus RNA not coding for protein or "non-coding RNA" (ncRNA) is now recognized as a key area for study of human diversity and disease (Dinger et al., 2008; Mattick and Makunin, 2005; Mendell and Olson, 2012; Tal and Tanguay, 2012).

MicroRNAs (miRNAs) are small ncRNA molecules of approximately 22 nucleotides in size that regulate the expression of genes by binding to the 3′-untranslated regions (3′ UTR) of target mRNA which inhibits protein translation or facilitates degradation of mRNA (Lewis et al., 2005). MiRNAs are encoded by genes that are transcribed from DNA distributed throughout the genome but not translated into protein. Over 1100 unique miRNAs have been identified with complementary binding for thousands of predicted mRNA targets. MicroRNAs are critical in development, differentially expressed in tissues, involved in viral infection processes and associated with oncogenesis (Mendell and Olson, 2012; Tal and Tanguay, 2012). Proliferating cells express mRNAs with shortened 3′ UTR and thus have fewer miRNA target sites (Sandberg et al., 2008). Different miRNAs are predicted to regulate the majority of human protein coding genes (Dinger et al., 2008; Mendell and Olson, 2012).

1.1. MiRNA in the nervous system

Multiple classes of ncRNAs are highly represented in the nervous system (Cao et al., 2006; Rogelj and Giese, 2004) emphasizing that nervous system development and function is heavily dependent on RNA regulatory networks with alterations resulting in neurological and psychiatric diseases (Mehler and Mattick, 2006). NcRNAs appear

Abbreviations: cDNA, complementary deoxyribonucleic acid; DNA, deoxyribonucleic acid; FDR, false discovery rate; GC content, guanine-cytosine content; mRNA, messenger ribonucleic acid; miRNA, micro ribonucleic acid; NIAAA, National Institute on Alcohol Abuse and Alcoholism; NICHD, National Institute of Child Health and Human Development; ncRNA, non-coding ribonucleic acid; PMI, post mortem interval; PCR, polymerase chain reaction; RIN, ribonucleic acid integrity number; RNA, ribonucleic acid; RMA, robust multichip average; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; scaRNA, small Cajal body-specific ribonucleic acid; snoRNA, small nucleolar ribonucleic acid; UCSC, University of California, Santa Cruz; UPGMA, un-weighted average distance; 3′UTR, 3′ untranslated regions.

☆ This investigation was supported by a grant from the Hubert & Richard Hanlon Trust, NICHD HD02528 and NIAAA K01-AA015935.

* Corresponding author at: Department of Psychiatry & Behavioral Sciences, MS 4015, University of Kansas Medical Center, 3901 Rainbow Blvd, Kansas City, KS 66160, USA. Tel.: +1 913 588 6473; fax: +1 913 588 1305.
E-mail address: amanzardo@kumc.edu (A.M. Manzardo).

0378-1119/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.05.052

to regulate the maintenance of mature neural traits and synaptic plasticity (Conaco et al., 2006; Sempere et al., 2004; Tal and Tanguay, 2012) and are heavily involved in synaptic function and memory formation (Ashraf and Kunes, 2006; Schratt et al., 2006). For example, dysregulation of miRNAs have been reported in association with Alzheimer disease, X-linked mental retardation, Parkinson disease, Tourette syndrome and schizophrenia (Ableson et al., 2005; Dostie et al., 2003; Krichevsky et al., 2003; Tal and Tanguay, 2012). However, less is known regarding miRNAs in the context of alcoholism.

1.2. MiRNA in alcoholism

The number of serious adverse health effects associated with alcoholic drinking would predict a wide range of miRNA disturbances in individuals with alcohol dependence relative to healthy controls. Exposure to alcohol has been associated with disturbances in miRNA expression in neuronal cells from rodents (Guo et al., 2012; Sathyan et al., 2007) and in developing zebrafish which correlate with neurobehavioral and skeletal abnormalities (Soares et al., 2012; Tal et al., 2012). The expression of several miRNAs (miR-9, miR-21, miR-153 and miR-335) was reportedly suppressed by alcohol in fetal cultures of mouse cerebral cortical neuroepithelium (Sathyan et al., 2007). Preliminary studies have identified a putative role for miR-9 in alcohol tolerance possibly mediated through decreased expression of the BK channel, a high conductance calcium and voltage-dependent potassium channel (Martin et al., 2008; Pietrzykowski et al., 2008). However, it is not known how the effects of chronic heavy alcohol use on miR-9 or other miRNA expression may differ in the adult human brain.

Lewohl et al. (2011) recently reported significant up-regulation, but no significant down-regulation, of 35 miRNA constructs in the prefrontal cortex of human alcoholics compared to non-alcoholic control subjects. Predicted targets of up-regulated miRNAs showed a high level of overlap with published cDNA expression disturbances. The functional classification of disturbed predicted targets included nervous system development, cellular adhesion, and cell–cell signaling. Our study seeks to identify and further characterize the nature of functional miRNA disturbances observed in the frontal cortex of alcoholic men and women relative to age, race and gender-matched controls in relationship to target gene expression using the latest microarray technology.

2. Methods

2.1. Samples

MicroRNA and mRNA (exon) expression profiles were obtained from total RNA isolated using the Qiagen (Qiagen Inc, Maryland) kit from post-mortem human frontal cortex (Brodman Area 9) of 9 alcoholics [6 males, 3 females; mean (±SD) age = 49.1 (±6.0) yrs, range 41–57 yrs] and 9 age and gender-matched control subjects [6 males, 3 females; mean (±SD) age = 50.0 (±6.6) yrs, range 37–56 yrs]. The pre-frontal cortex was selected for study due to its role in the regulation of motivated behaviors, impulse dysregulation in addiction and as a region of the brain particularly susceptible to the effects of long-term alcohol abuse (Ross and Peselow, 2009). The average RNA integrity number (RIN) was 6 for the alcoholics and 5 for the controls and considered adequate for microarray analysis. The gender composition of the sample reflects the sex ratio distribution found in the general population of alcoholics. Samples were procured from the New South Wales Tissue Resource Centre (Sydney, Australia) and collected according to a standardized protocol (Sheedy et al., 2008) in compliance with ethical guidelines established by the Sydney South West Area Health Service Human Ethics Committee (X03-0074). Informed written consent was obtained from the nearest living relative. The mean (±SD) post-mortem interval (PMI) for our subjects was 26.3 (±9.6) hours with a range of 13 to 43 h. The mean (±SD) sample pH was 6.6 (±0.22). All samples tested were negative for viral hepatitis and for the human immunodeficiency virus.

All subjects were of European descent and alcohol dependent subjects met the criteria described in the Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition and National Health and Medical Research Council/World Health Organization criteria. Control subjects were social drinkers (non-abstainers for alcohol use) and did not meet criteria for alcohol abuse or dependence. The average estimated duration of alcohol dependence for case subjects was 19.5 (±8.1) years (range 10–30 years). The individual causes of death varied across participants with the most common causes due to cardiovascular and respiratory problems or infection. Direct alcohol toxicity or overdose was indicated in two deaths of alcoholic subjects. Family history of alcohol problems was either negative or unknown for all subjects.

2.2. Microarrays

The GeneChip miRNA 2.0 Array (Affymetrix, Inc.; Santa Clara, CA) was used to examine miRNA disturbances in Alcohol Dependent subjects relative to age and gender-matched controls. The array specifications included the following information: *Array type* — GeneChip miRNA 2.0; *source* — Sanger miBase miRNA (http://microrna.sanger.ac.uk) for mature and pre-miRNA; snoRNABase (www.snorna.biotoul.fr/coordinates.php) and Ensembl Archive (www.ensembl.org/biomart/martview) for small nucleolar RNA (snoRNA) and small Cajal body-specific RNA (scaRNA); *build* — all probe locations used the human genome reference GRCh36/hg19 assembly (http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19); *probe length* — 25 mer or less. The GeneChip miRNA 2.0 array uses 20,287 total probe-sets to provide 100% coverage of miRBase v15 (15,644 probe-sets); 2334 snoRNA and scaRNA; and 2202 unique pre-miRNA hairpin sequences from 131 organisms. The human probe-set provides coverage for 1105 mature miRNA; 1121 pre-miRNA; 2302 snoRNA; and 32 scaRNA and hybridization with processing was performed at the same time using the same batch of commercially generated microarrays. The scope of the present study was limited to an analysis of human miRNA disturbances excluding snoRNA and scaRNA.

The Human Exon 1.0 ST (sense target) Array (Affymetrix, Inc.; Santa Clara, CA) was used to identify disturbances in mRNA that inversely correlate with miRNA changes (i.e., increased expression of specific miRNAs should show decreased expression of the predicted target mRNAs) generated from miRNA arrays. The co-expression patterns of the predicted target mRNAs were used to assess the functional impact of observed miRNA disturbances obtained from the same RNA source, a practice gaining acceptance as an experimental validation method merging high-throughput techniques with existing gene-specific assays including advanced microarray screening as performed in our study in place of conventional quantitative RT-PCR (Vergoulis et al., 2012; Vlachos et al., 2012). For example, comparison of the number of targets per experimental validation method for the TarBase 5.0 and TarBase 6.0 prediction software programs indicates an increasing use of high-throughput techniques including microarray platforms which currently follows only sequencing as the most widely used techniques for this purpose (Vergoulis et al., 2012). Hence, the selection of human exon arrays for experimental validation of miRNA disturbances in our study. Array specifications included the following information: *Array type* — Human Exon 1.0 ST; *source* — cDNA-based content including the more established human RefSeq mRNAs, GenBank® mRNAs, and ESTs from dbEST. Additional annotations were created by mapping syntenic cDNAs to the human, mouse, and rat genomes using genome synteny maps from the UCSC Genome Bioinformatics group. Predicted gene structure sequences from GENSCAN; Ensembl; Vega; geneid and sgp; TWINSCAN; Exoniphy; microRNA Registry; MITOMAP; and structural RNA predictions; *build* — all probe locations used the human genome reference GRCh36/hg19 assembly (http://genome.ucsc.edu/cgi-bin/hgGateway?db=hg19): *probe length* — 25 mer or greater. The Human