Journal of Psychiatric Research 99 (2018) 39–49

Contents lists available at ScienceDirect

Journal of Psychiatric Research

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

Examining redox modulation pathways in the post-mortem frontal cortex in patients with bipolar disorder through data mining of microRNA expression datasets

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ARTICLE INFO

Keywords:
Bipolar disorder
microRNA
Redox modulation
Data mining
Oxidative stress
Mitochondria

ABSTRACT

The etiology of redox (reduction and oxidation) alterations in bipolar disorder (BD) is largely unknown. To explore whether microRNAs targeting redox enzymes may have a role in BD, we examined 3 frontal cortex microRNA expression datasets (Perkins [2007], Vladimirov [2009], and Miller [2009]; N for BD = 30–36 per dataset, N for controls = 28–34 per dataset) from the Stanley Neuropathology Consortium. Each dataset was analyzed separately because they were generated using different high-throughput platforms. Following the selection of only redox modulator-targeting microRNAs, microRNAs in the top 10th percentile in feature selection could together discriminate BD and controls at a greater frequency than expected by chance in classification analysis. In pathway enrichment analysis of all three datasets, these classifying microRNAs targeted the cellular nitrogen compound metabolic process pathway, which includes redox enzymes of the mitochondrial electron transport chain and the glutathione system. To see if this pathway would still emerge as significant if all microRNAs (not just redox-targeting) were analyzed, all analyses were repeated with the complete set of microRNAs. Cellular nitrogen compound metabolic process pathway was enriched in all 3 datasets in this analysis as well, demonstrating that preselection of redox microRNAs was not a requirement to identify this pathway for the discrimination of BD and controls. While preliminary, our findings suggest that microRNAs that target redox enzymes in this pathway may be good candidates for the exploration of causative factors contributing to redox alterations in BD. Future studies validating these findings in a separate set of central and peripheral samples are warranted.

1. Introduction

Reactive oxygen species (ROS) produced by the mitochondrial electron transport chain (mETC) and other oxidants cause functional alterations (Jones, 2010). The production and neutralization of ROS (i.e. redox modulation) need to be in balance for the maintenance of cellular function (Cross and Templeton, 2006; Jones, 2010).

Bipolar disorder (BD) is a complex disorder with multiple pathways contributing to its etiology, including apoptosis, dysregulation of neurotrophins and synaptic alterations (Berk et al., 2011, 2013; Kapczinski et al., 2011). Impairments in redox modulation are among the most consistent findings in BD, including increased lipid peroxidation (Versace et al., 2013) and altered oxidation and nitration of proteins (Andreazza et al., 2013; Kim et al., 2014). However, the etiology of these alterations remains elusive.

Over half of all coding genes are regulated by microRNAs (miRNAs) (Witkos et al., 2011), which can alter the expression of their target genes (Issler and Chen, 2015; Valinezhad Orang et al., 2014). A recent study showed that a number of miRNAs altered in major depression target glutathione, the main antioxidant in the brain (Bocchio-Chiavetto et al., 2013). Furthermore, alterations in miRNA levels have been reported in BD (Banigan et al., 2013; Smalheiser et al., 2014).

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https://doi.org/10.1016/j.jpsychires.2018.01.011
Received 20 September 2017; Received in revised form 12 December 2017; Accepted 18 January 2018

These findings suggest that miRNAs may contribute to changes in the expression of redox modulators in BD.

To investigate this possibility, we analyzed publicly available datasets containing miRNA expression levels from post-mortem brain samples from patients with BD. Depositing high-throughput data is becoming common upon being accepted for publication. Due to the use of different platforms and analytical techniques, however, it is often not practical or useful to combine datasets from different studies for analysis. Platforms may vary in their use of probes, as well as limits of sensitivity, resulting in interstudy differences in identified miRNAs, expression levels, and between-group differences (Callari et al., 2012; Leshkowitz et al., 2013). However, it would be logical to assume that each assay used to investigate samples likely derives biologically meaningful data if the data are of adequate quality, as measured by available quality metrics. While specific miRNAs of interest may differ between datasets, data mining methods can be deployed to assess whether miRNAs emerging from different datasets belong to the same functional pathways. This provides an opportunity to identify pathways that may be important for the pathophysiology of a disease, creating opportunities for future studies. Hence, we aimed to identify miRNAs that target redox pathway elements, and then explored whether these could be implicated in the pathophysiology of BD. Using machine learning algorithms, we analyzed 3 datasets that were obtained using different platforms but from the same cohort of post-mortem brain samples. We hypothesized that if there is an overlap in the implicated pathways among the 3 datasets (but not necessarily an overlap in the specific miRNAs), and if these pathways include redox enzymes known to be altered in BD, this may suggest the involvement of miRNAs in redox alterations in BD.

2. Material and methods

2.1. Datasets

Three datasets (Perkins (2007), Vladimirov (2009), and Miller (2009)) of miRNA expression values derived from the post-mortem frontal cortex from patients with BD and unaffected controls were obtained from the Array Collection at the Stanley Neuropathology Consortium Integrative Database (http://sncid.stanleyresearch.org/; Stanley Medical Research Institute (SMRI)) (Kim and Webster, 2010). Each dataset was derived from the same brain samples using different platforms (Agilent miRNA microarray, Taqman liquid density array, ORB human Sanger 12 plus miRNA array, respectively). As expected, the list of miRNAs measured were different between the 3 datasets. We used the Venn Diagrams Tool from The Bioinformatics and Evolutionary Genomics Group (http://bioinformatics.psb.ugent.be/webtools/Venn/) to find that only 230 miRNAs were present in all 3 datasets (Fig. 1). Therefore, each dataset was analyzed separately. To the best of our knowledge, the Vladimirov (Kim et al., 2010) and Miller (Miller et al., 2012) datasets have been used to produce 1 publication each. There were no overlaps in the miRNAs identified in the two published studies for discriminating BD and controls. Using SPSS24.0 (IBM), the Kruskal-Wallis H test was performed to see if there was a between-group difference in post-mortem interval, brain pH, and age in each of the datasets. Perkins, Vladimirov, and Miller datasets will be referred to as A, B, and C hereafter, respectively.

2.2. Tarbase redox modulator target search for miRNA datasets

All experimentally verified gene targets of the miRNAs that were above the detection threshold in each dataset were identified using Tarbase v.7.0, as this was felt to be a more conservative approach than identifying targets based on prediction algorithms (Vlachos et al., 2015). Redox modulators were defined to include subunits of mETC, nitric oxide synthase, and antioxidant enzymes in the glutathione, thioredoxin, superoxide dismutase, glutaredoxin, catalase, and peroxidase pathways (Supplementary Table 1). Expression levels for these redox modulator-targeting miRNAs were extracted from each of the original datasets, resulting in three reduced datasets. These reduced datasets for Perkins, Vladimirov, and Miller will also be referred to as D, E, and F from hereafter, respectively.

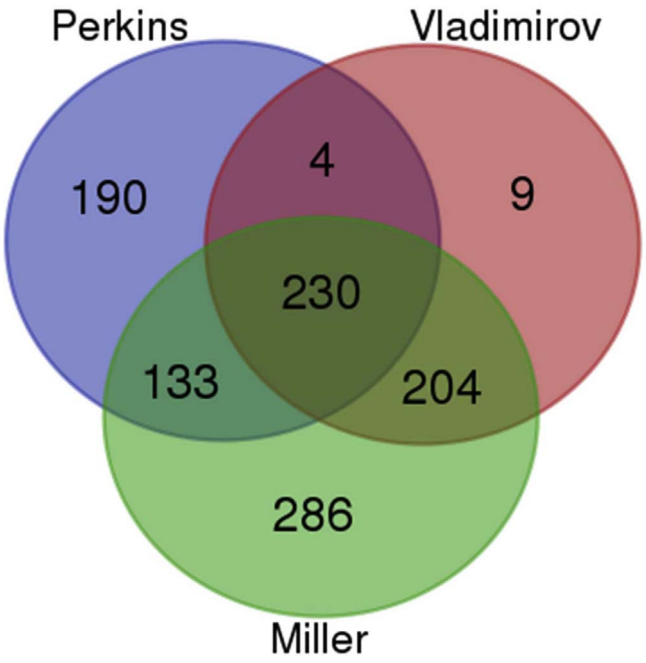

Fig. 1. Venn diagram showing differences and overlap between the miRNAs measured in the 3 miRNA datasets (Perkins, Vladimirov, Miller) from SNCID.

2.3. Rank feature selection and classification analysis of redox miRNAs

Following selection of miRNAs that are likely to impact redox pathways, outliers were removed by calculating the mean correlation of the miRNA expression profile for each subject with all other subjects. While individual miRNA levels may differ among subjects due to disease, all miRNAs from one subject, as a group, should correlate with all miRNAs from other subjects, unless there is a technical error. Using the IQR (interquartile range) outlier labeling method, outliers were identified and removed (Hoaglin and Iglewicz, 1987). Preprocessing was performed for all 3 datasets. While the Perkins (2007) dataset has not been published, we ensured its adequacy for miRNA expression analysis after preprocessing. Prior to preprocessing, subject information was identical for all datasets, which were derived from the same subjects (Supplementary Table 2). Subject information after the removal of outliers can be found in Table 1. Correlation between miRNAs and lifetime antipsychotic dose, and between miRNAs and duration of illness were determined using the Spearman's rank-order correlation test (significant if $p < 0.05$). Kruskal-Wallis H test was used to explore if miRNA levels varied between patients who committed suicide and patients who did not. Rank feature selection with 10-fold validation was performed using a previously published method (Ren et al., 2017) with the Statistics and Machine Learning Toolbox™ in Matlab2016b. Feature selection analysis ranked the miRNAs according to their ability to differentiate between BD and controls. For 10-fold validation, rank feature selection was performed 10 times, where each run consisted of 90% of the subjects that were randomly selected. This was repeated 10 times, and the resulting rankings were then averaged to produce the final ranking. Feature selection was performed first to identify a set of miRNAs with greater predictive accuracy in terms of differentiating between BD and controls, and second, to allow us to focus only on the miRNAs with differentiating ability for further analyses. Classification analysis was then performed to quantify the prediction accuracy of this