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POTENTIAL USE OF MICRORNA FOR MONITORING THERAPEUTIC RESPONSE TO ANTIDEPRESSANTS

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ABSTRACT

Major depressive disorder (MDD) is a serious and common psychiatric disorder that affects millions of people worldwide. The most common treatment methods for MDD are antidepressant drugs, many of which act by regulating monoamines by inhibiting pre-synaptic reuptake and/or by modulating monoamine receptors. Despite advances in antidepressants and other treatment options, therapy is often based on subjective decisions made by the physician. Moreover, it requires time to determine treatment outcome and to define whether the prescribed treatment is effective. Biomarkers may help identify individuals with MDD who are more likely to respond to specific antidepressant treatment and may thus provide more objectivity in treatment decision making. MicroRNA as biomarkers of antidepressant response has engendered substantial enthusiasm. In this review, we give a detailed overview of biomarkers, particularly the major studies that have investigated microRNA in relationship to antidepressant treatment response.

Key Points

Several microRNAs (miRNAs) demonstrate translational evidence of dysregulation during major depressive disorder and change during antidepressant treatment. These miRNAs could be defined as predictive or mediator biomarkers of antidepressant response. However, no miRNA has yet been defined as a validated biomarker to predict or monitor antidepressant response, and much larger clinical studies are needed.

INTRODUCTION

Major depressive disorder (MDD) is a serious and common psychiatric disorder characterized by alteration of mood and emotional process. Symptoms include anhedonia, sleep disorders, sexual and appetite disturbances, psycho-motor activity alteration and neuropsychological functioning disturbances (1). It is also one of the key risk factors for suicide attempts and death by suicide in the general population (2, 3).

More than 30 different antidepressant drugs, belonging to different pharmacological classes with several distinct pharmacodynamic profiles, are available in developed countries. Despite the wealth of treatment strategies available, patients with depression often do not experience symptomatic remission or treatment response, even after trialing several medications (4). The large majority of antidepressants act via monoamine regulation by inhibiting their pre-synaptic recapture and/or by modulating monoamine receptors. However, the exact mechanisms by which they elicit antidepressant response are still unknown. The most popular antidepressants are selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), and other antidepressant treatments may act through melatonin agonism, dopamine regulation or monoamine oxidase inhibition (5). These antidepressants exhibit different pharmacokinetic properties and side-effect profiles; approximately half are considered first-line treatments (5). Alternatives to conventional antidepressant pharmacotherapy include psychotherapy and somatic treatments (including brain stimulation and physical exertion). In addition, other pharmacological treatments (i.e. lithium or atypical antipsychotics) can be used as augmenting agents in cases of resistance to classical antidepressants, and new pharmacological options such as ketamine are in development. However, which treatment strategy leads to a

better response is unclear (6). Consequently, and despite important improvements in treatment recommendations, which can now include risk–benefit ratios, recommendations for major depressive episodes [MDE] with specific characteristics and for specific populations, treatment decision making ultimately involves clinician experience and patient perceptions, which are subjective.

There are several reasons why antidepressant treatment may be ineffective. The choice of antidepressant, and optimizing that choice, represents a major issue, and other challenges include improving medication adherence. While the patient's clinical profile and characteristics appear to hold no answers to this question, biomarkers may help better identify patient subpopulations and individuals more likely to respond to a given treatment option (7).

The US FDA defines a biomarker as a defined characteristic that is measured as an indicator of normal or abnormal biological process, or the response/resistance to an exposure or intervention, including pharmacological treatment (8). A biomarker may be molecular, biochemical, radiological, and so on. In this review, we discuss molecular biomarkers, particularly the growing research investigating microRNAs (miRNAs) as potential biomarkers of response to antidepressant treatment. To conduct this review, we used the PubMed database with the following keywords individually and in combination: miRNA, animal model, gene expression regulation, major depressive episode (MDE), major depressive disorder (MDD) and antidepressant response. We included all relevant research articles focusing on miRNA variations in humans or in animal models of depression.

Types of Biomarkers and Measurement Properties

Biomarkers are typically conceptualized and investigated as predictors or mediators. Predictor biomarkers define baseline characteristics that predict illness outcomes (e.g. chronic course) or treatment response. Mediator biomarkers are characteristics that change during the course of illness or as a result of treatment. Mediator biomarkers may also be predictor biomarkers and, as such, may help elucidate the biological mechanisms underlying antidepressant effects and in the development of new treatment avenues (7, 9, 10).

The identification and validation of biomarkers is a complex process that is not limited to the description of mean group differences (e.g. lower level of expression of a specific miRNA between responders and non-responders before treatment) and requires several important steps (Table 1) (11-13). Biological or analytical validity must first be confirmed. Measurement properties (precision and level of detection) must be verified. Inter-experimental and intra-individual variability must be low and can be measured via coefficient of variation (CV; standard deviation/mean × 100). Although there is no gold standard value for CV, a CV < 15% is generally assumed to be reliable. Intra-class correlation coefficient (ICC), which reflects the relative balance of between- and within-person variance components, can also be used. An ICC >0.75 is usually considered a good threshold. Next, the clinical validity or overall accuracy of a biomarker must be tested using receiver operating characteristic (ROC) curve analysis and/or logistic regression, along with a Bayesian approach (14). These analyses enable the description of biomarker characteristics such as sensitivity, specificity, positive and negative predictive value, and accuracy of the test, area under the curve (AUC) and likelihood ratios. These characteristics are crucial because statistically significant mean differences do not imply clinical

relevance. Biomarker characteristics are based on the relationship between findings from biomarkers (positive or negative test) and the actual classification of a subject given a phenotype (true or false) as described in a 2 × 2 contingency table (14, 15). Sensitivity is the ability of the biomarker to detect a true-positive test among all true phenotypes (i.e. sensitivity = true positive/[true positive + false negative]). Specificity is the ability of the biomarker to detect a true-negative test among all false phenotypes (i.e. specificity = true negative/[true negative + false positive]). Positive and negative predictive values are based on the relationship between sensibility, specificity and prevalence of the phenotype. Moreover, accuracy of the biomarker could be defined as the proportion of patients correctly classified (i.e. sum of the true-positive and true-negative tests) based on a 2 × 2 contingency table (15). However, unlike qualitative variables, quantitative biomarkers such as miRNA do not directly allow a 2 × 2 contingency table. Logistic regression determines the proportion of patients correctly classified using the biomarker and odds ratios (OR) associated with the biomarker. ORs could be interpreted as the variation of the probability of correct classification of a patient when the biomarker value is increased by one unit. Moreover, a threshold of the biomarker results could be determined by analysing the ROC curve, which shows the relationship between sensitivity (Y axis) and '1 − specificity' (X axis) for each value or different cut-off of the biomarker from a study. Consequently, the calculation of the higher Youden index (Y = sensitivity + [specificity − 1]) represents the nearest point of the curve from the left upper angle and determines a threshold value for the quantitative biomarker. The AUC of a test is also computed by ROC curve analysis. Biomarkers developed in daily clinical practice must demonstrate at least AUC > 0.75 to be considered to have reasonable diagnostic properties, an AUC > 0.90 is usually required (14, 16). Likelihood ratios (LHRs) correspond to the ratios of the probability of a positive or negative test

result in one group versus another (e.g. responders vs. non-responders) for a positive or negative test (positive LHR and negative LHR, respectively) (17) and can be calculated as follows: LHR + = sensitivity/(1 − specificity) and LHR– = (1 − sensitivity)/specificity. Interestingly, LHRs allow the calculation of the change in predictive ability when using the new biomarker compared with usual practice. In fact, the odds of the phenotype after using the biomarker = LHR + × pre-test odds, where pre-test odds is the probability of the phenotype (e.g. antidepressant response) based on the current epidemiological knowledge or based on the current best predictive tool used without the biomarker. For example, if antidepressant response occurs in 60% of patients (odds = 1.5), and we develop a biomarker with an LHR+ = 2, using the new biomarker in our treatment plan will allow us to obtain a new probability of response in our population of 75% (post-test probability = (1.5 × 2)/([1.5 × 2] + 1). The post-test probability could also be determined without odds calculation, using a Fagan nomogram with pre-test probability and LHR (17). In biomarkers developed in clinical practice, LHR+ needs to be >5 to achieve good diagnostic value, and a LHR+ >10 is commonly recommended (14). The potential advantage of LHRs over positive and negative predictive values is the theoretical independence of LHRs to the prevalence of the predicted phenotype. Consequently, these characteristics are more easily adapted when no robust knowledge of phenotype/outcome prevalence is available and/or when the prevalence is too sensitive to several factors between different populations and therefore too heterogeneous between these populations.

The clinical utility of a biomarker must also be proven. Although clinical validity demonstrates that the biomarker predicts response, clinical utility refers to the capacity of the biomarker to actually improve response rate. To estimate clinical utility, the biomarker should be compared

against a gold standard procedure for prediction. However, in the absence of any gold standard measure in psychiatry, this characteristic is difficult to estimate, and one could assume a biomarker with good clinical validity may have good clinical utility in psychiatric clinical practice. However, interventional clinical efficacy studies are needed to clearly demonstrate clinical utility (i.e. how the biomarker improves treatment efficacy by predicting treatment response). These studies should ideally be conducted using randomized controlled trials. Moreover, it would be important to test the biomarker in other populations to explore the specificity of the findings.

The next step involves analysis of clinical usefulness: can the biomarker be used in daily practice? The final step is to conduct health economics studies to document the savings associated with using the biomarker. It is worth noting that, if a biomarker is discovered using a hypothesis-free approach or without a strong biological rationale, biological plausibility could also be an important supplementary step in the development of this biomarker (12). Functional analysis, including animal models, cellular models and all other procedures, could improve understanding of and describe a causal link between pathophysiology and the biomarker.

While no biomarker currently meets all these criteria and none can therefore be recommended to help diagnosis and improve treatment in psychiatric practice (5), recent advances in molecular biology raise hope that this may soon change. A growing number of studies have been suggesting that miRNAs may be excellent candidates as biomarkers, particularly as interesting biomarkers of antidepressant response.

MicroRNA (miRNA) Processing and Function

miRNA are single-stranded non-coding RNAs that are 17–22 nucleotides long and usually transcribed by RNA polymerase II (18, 19). Transcription of an miRNA gene results in a primary miRNA (pri-miRNA) transcript. The pri-miRNA is typically 1 kb long and exhibits a 33–35 base-pair stem structure with a terminal loop and single-stranded RNA strands on both the 5′ end and 3′ end of the stem-loop structure. The pri-miRNA is further processed in the nucleus by Drosha, a class 2 RNase III enzyme. Drosha cleaves the pri-miRNA, releasing a 60- to 65-nucleotide hairpin-structured RNA called the precursor miRNA (pre-miRNA) (20). Pre-miRNAs are then transported out of the nucleus via exportin-5 and further processed into mature miRNAs by Dicer (an endonuclease cytoplasmic RNase III enzyme). After the 17- to 22-nucleotide mature miRNA is released from the pre-miRNA, it is loaded into an Argonaute (AGO) family protein complex forming an effector complex called RNA-induced silencing complex (RISC). Usually one strand of the miRNA (passenger strand/miRNA*) is degraded, while the other miRNA strand (guide strand/miRNA) remains bound to the AGO protein (18, 19).

MicroRNA have two key roles in the regulation of messenger RNA (mRNA). They act by inducing either degradation or translational silencing of the mRNA they target. Target recognition is primarily determined by a stretch of six nucleotides near the 5′ end of the miRNA, known as the seed region. miRNA primarily bind via their seed region to mRNA at the 3′ untranslated region (UTR) (19). Moreover, because of the short length of complementarity, a single miRNA can be predicted to target multiple gene transcripts, and a single transcript can be targeted by several miRNAs. Thus, individual miRNAs are believed to form a complex network with other miRNAs as well as other non-coding RNA species to regulate gene expression at the

post-transcription level. Over the years, miRNA have demonstrated a clear role and importance as their own class of gene-regulatory molecules and are thus believed to play a major role in disease aetiology.

Moreover, miRNAs may be excellent biomarkers because they are easily measurable with a diverse range of robust techniques such as small-RNA sequencing, microarrays and quantitative polymerase chain reaction (PCR). In particular, miRNAs are released by cells and circulate in biological fluids such as blood in exosomes, acting as possible signals in cell-to-cell communication (21). This property makes them especially interesting candidates for investigation as biomarkers. As such, it is possible that circulating miRNAs in peripheral blood may reflect miRNA expression/dysfunction in the brain.

miRNA in the Central Nervous System (CNS)

miRNAs are involved in multiple stages of brain development, including neural stem cell proliferation, differentiation, migration, dendrite complexity and axonal outgrowth. Moreover, miRNA have been shown to be involved both in early embryonic development and in adult neurogenesis in mammals. In mice, conditional knock-out of Dicer at late embryonic stages affected neuron migration in the cortex (22). Moreover, Dicer depletion has also been shown to affect proliferation, cell death, migration and differentiation during corticogenesis in the developing brain (23). A separate study identified over 100 distinct miRNA expressed in olfactory tissue, which undergoes neurogenesis throughout late adulthood (24). Dicer knock-out in olfactory progenitor cells resulted in ablation of terminal differentiation of the olfactory progenitor pool into mature olfactory neurons (24).

Several studies have also shown miRNAs to be involved in synaptic plasticity, the strengthening and weakening of synaptic connections involved in learning and memory. Reduction in dendritic spines in rat hippocampal neurons was shown to be associated with miR-138 and miR-134. Lim-domain-containing protein kinase I (Limk1) is a regulator of actin filaments, and knock-out studies in mice have shown abnormalities in dendritic spine morphology (25). More specifically, miRNA-134 negatively regulates dendritic spines by reducing levels of Limk1 mRNA (26). Furthermore, other independent studies have shown miR-132 and miR-9 reduce dendritic length and arborization, respectively. miR-132 inhibits p250 GTPase-activating protein to induce neurite sprouting in cortical neurons (27-29). Interestingly, this miRNA has been described as being downregulated in rats submitted to learned helplessness paradigms (30). miR-9 is brain specific and represses nuclear receptor TLX, which is involved in maintaining neural stem cells in a self-renewable state, during neural stem cell differentiation (31). miR-9 inhibits TLX, resulting in decreased neural stem cell proliferation and increased neural differentiation (31). This study showed that the miR-9-TLX regulatory loop plays a role in determining neural stem cell fate. miR-9 has also been reported to be involved in axon projection. miR-9 was found to locally suppress microtubule-associated protein 1B (Map1b) in axons, which resulted in a reduction of axon length (32). These studies give sufficient evidence that miRNAs play a critical role throughout the development of the CNS as well as maintaining CNS function throughout adulthood. As these functions have been related to the pathophysiology of depression, these results give weight to the hypotheses that miRNAs could be involved in depression.

miRNAs and Depression

Several studies have explored potential variations of miRNA expression in depression in post-mortem brain samples or peripheral tissue from patients with MDD as well as in animal models of depression. Table 2 lists miRNAs with several lines of evidence of association with depression and antidepressant response. One of the first studies to profile miRNA in the context of depression and suicide in humans looked at global miRNA expression (miRNome) in the prefrontal cortex (PFC). This study found a ~17% global downregulation of miRNA in subjects with depression who died by suicide compared with controls (33). Looking at the miRNAs individually, 21 miRNAs showed significant downregulation. Target predictions performed for the downregulated miRNAs revealed several mRNA targets, including vascular endothelial growth factor (VEGF), anti-apoptotic protein B cell/lymphoma 2 (BCL2) and DNA methyltransferase 3B (DNMT3B), which were previously implicated in MDD and further explored in this study. More precisely, miR-20b, miR-20a, miR-34a and miR-34b are predicted to target VEGF, a growth factor associated with depression that has been shown to be significantly elevated in peripheral blood of humans with MDD compared with controls (33). Interestingly, among the miRNAs described in this study, miR-101b has also been described to be downregulated in the PFC in the Flinders Sensitive Line rat, a genetic model of depression (34). An additional miRNome study by the same group found miR-508-3p and miR-152-3p to be downregulated in post-mortem PFC tissue from subjects with MDD (35).

The miRNome has also been profiled in the peripheral blood of humans with MDD. Using a microarray assay, Fan et al. (36) found nine upregulated and one downregulated miRNA. Validation using quantitative reverse transcription PCR (qRT-PCR) in a distinct MDD group indicated that miR-26b, miR-4485, miR-1972, miR-4498 and miRNA-4743 were significantly

upregulated in subjects with MDD compared with in controls (36). An additional study also profiled miRNA expression in peripheral blood using microarrays and found seven miRNAs that showed differential expression in patients with MDD compared with controls. Four miRNAs (let-7a-5p, let-7d-5p, let-7f-5p and miR-1915-3p) were significantly downregulated, and three miRNAs (miR-199a-5p, miR-24-3p and miR-425-3p) were significantly upregulated, in subjects with MDD (37). Let-7a-5p, let-7d-5p, let-7f-5-, miR-24-3p and miR-425-3p were validated using qRT-PCR and confirmed the results observed from the microarray.

Two recent studies described translational evidence for the involvement of miRNA in MDD. One study demonstrated that miR-124-3p is associated with MDD on the basis of an animal model of depression (induced by exogenous corticosterone administration), human post-mortem brain study and peripheral serum study in medication-free patients with MDD (38). The other study demonstrated the involvement of miR-218 in stress-related disorder. The authors described a reduction in the expression of miR-218 in post-mortem subjects who died by suicide and a negative correlation between the level of expression of miR-218 and a candidate gene, DCC (disrupt in colorectal cancer) (39). The author demonstrated translational validation of their findings in susceptible mice with chronic stress-induced social avoidance.

Finally, two other studies described miRNA variations in MDD (37, 40). The first described miRNA variations associated with MDD in the anterior cingulate cortex. The authors described dysregulation that did not survive correction for multiple testing (40). The second used a micro-array approach and described variations in the expression of several miRNAs in the blood of patients with MDD compared with healthy subjects (37). Interestingly, this study highlighted that

some miRNAs could be specific to MDD and others could also be dysregulated in bipolar disorder.

These studies indicate that miRNAs are involved in many aspects of the CNS as well as MDD aetiology. In the next section, we discuss the potential of miRNAs as biomarkers for MDD and/or antidepressant effect.

miRNAs as Biomarkers for Antidepressant Response

Despite a growing interest in the investigation of miRNAs in the pathophysiology of MDD, very few studies have investigated miRNAs as biomarkers of antidepressant response. Table 3 provides a summary of miRNAs with at least two lines of evidence of association with MDD or antidepressant response. Results come mainly from a few different prospective studies that included samples from patients with MDD. These studies used a hypothesis-driven approach according to literature, or a whole-genome hypothesis approach.

Baudry et al. (41) conducted an elegant rodent study and identified miR-16 as an important regulator of the serotonin transporter; they also found it mediated therapeutic response with fluoxetine, an SSRI. A subsequent study by the same group (42) indicated that hippocampal adult neurogenesis, thought to explain antidepressant response, is also mediated by miR-16. On the basis of these results, one study (43) demonstrated that miR-16 was downregulated in the cerebrospinal fluid but not the blood of patients with depression compared with healthy controls. Although this study provided more evidence to potentially implicate miR-16 in the pathophysiology of depression, it did not provide new information about the implication of this

miRNA in antidepressant response. Moreover, several studies in humans have not found evidence suggesting that variations in mir-16 in peripheral blood mononuclear cells (PBMCs) or whole blood correlates with antidepressant treatment response, suggesting that miR-16 may be associated with MDD and antidepressant response in a tissue-specific manner (44, 45).

We conducted a genome-wide miRNA expression study in the PFC of depressed individuals compared with psychiatrically healthy controls and found that miR-1202, a primate-specific and brain-enriched miRNA, was significantly downregulated in MDD (46). miR-1202 regulates the glutamate metabotropic receptor 4 (GRM4), a class III glutamatergic receptor that was proposed as a new target for antidepressant development (46). Studies in an independent sample confirmed the initial results and suggested that antidepressant treatment increased levels of miR-1202 (46). Subsequent studies using cell assays indicated that chronic treatment with at least two antidepressants, imipramine and citalopram, increased miR-1202 and decreased GRM4 levels (46). Consistent with this observation, a prospective trial in patients with depression indicated that baseline miR-1202 expression was lower in responders to citalopram, whereas non-responders demonstrated no change in miR-1202 expression compared with healthy control subjects (46). Interestingly, we also found evidence that variations in miR-1202 were correlated with variations in depressive symptoms as a result of citalopram treatment as measured by the Hamilton depression scale (HAM-D) (46). Moreover, a recent study focusing on a genetic variant in the GRM4 gene within the binding site of miR-1202 provided additional evidence for an association between miR-1202, GRM4 and MDD (47). Finally, we recently used a brain imaging study to demonstrate the relevance of testing miR-1202 levels in blood in relation to brain functioning. In particular, we found that variations in miR-1202 levels during

antidepressant treatment was correlated with changes in brain activity, including pre-frontal and cingulate cortices (48).

Issler et al. (45) conducted an interesting study investigating miRNAs in depression and treatment response and demonstrated that miR-135a had a lower expression profile in both the brain and the blood of patients with MDD. On the basis of animal experiments, the authors found miR-135a was upregulated by imipramine and fluoxetine but found no effect from reboxetine, a specific noradrenergic reuptake inhibitor. However, they did not find an effect in the whole blood of patients receiving escitalopram treatment for 12 weeks. Intriguingly, patients treated with psychotherapy within the same randomized trial demonstrated an upregulation of miR-135a.

In a small naturalistic study, Belzeaux et al. (49) also described that several miRNAs were deregulated during antidepressant response in PBMCs (nine patients treated as usual with various antidepressants): miR-20b, miR-433, miR-409, miR-485, miR-133a, miR-145 and miR-331.

In another naturalistic study, He et al. (50) demonstrated that miR-124 was over-expressed in the PBMCs of medication-free patients with MDD (n = 32) compared with those of healthy controls (n = 30) and downregulated after various antidepressant treatments from different pharmacological classes (n = 30) in accordance to response status. miR-124 variation across time was correlated with variations in depressive symptoms according to the HAM-D.

Gururajan et al. (44) demonstrated that let-7b expression was lower in patients with treatment-resistant depression (n = 40) than in healthy controls but was not predictive of response to, nor

affected by, electroconvulsive therapy (n = 23) or ketamine (n = 13). In this study, the authors also focused on miR-182, miR-223 and miR-451, which have been previously associated with MDE or with depressive phenotype in animal models of depression (51-53). They described no significant differences in the expression levels of these miRNA between treatment-resistant depression and healthy controls or between responders and non-responders to electroconvulsive therapy or ketamine. Using weighted correlation network analysis and bioinformatic prediction with Topp-Fun tools (54), we described several miRNAs that could be associated with antidepressant response. On the basis of gene co-expression analyses, we identified sets of correlated mRNA, called 'modules', that were associated with citalopram response and identified potential miRNAs that could regulate these mRNA. Results were replicated in another cohort of patients also treated with citalopram as well as in a naturalistic cohort of patients treated as usual (54). Among them, miR-92 and miR-1271 were interesting candidates. It is worth noting that, in this study, miR-135 and miR-16 were associated with antidepressant response modules as well as other non-specific modules (54).

Finally, a recent small study (55) that included five antidepressant-free patients demonstrated variations in miRNA levels in plasma after antidepressant treatment. All patients experienced remission after 12 weeks of escitalopram treatment. The authors detected 222 miRNAs using miScript miRNA PCR Array Human miRNome (Qiagen), among which, they described 40 potentially dysregulated miRNA during antidepressant treatment. However, the authors did not apply any correction for multiple testing.

Future Directions

The studies discussed in this review highlight specific miRNAs associated with depression or antidepressant response. While interesting, they provide only preliminary evidence that specific miRNA may act as biomarkers. As described in Table 2, few miRNAs were associated with MDD and antidepressant response with several lines of evidence. Moreover, biomarker research involving miRNA is still in its infancy. More precisely, efforts must be made to describe the clinical validity and clinical utility of miRNAs as biomarkers of MDD and antidepressant response following the steps described in Table 1. Moreover, we need to understand how specific the results are to one antidepressant over another, or even how generalizable they are to other non-pharmacological treatment options. To date, no study has included a comparison between two antidepressants or two therapeutic methods. No randomized controlled trials have tested the efficacy of using such biomarkers in comparison with treatment as usual. Meta-analysis would be useful to synthesize previous findings and identify the more robust results; however, we think the study design and methodology of current trials are too heterogeneous, and sample sizes are too small, to enable such analyses.

Not only do we need to replicate these results in larger samples and conduct studies to provide higher levels of evidence for miRNAs as biomarkers of treatment response, we also need to better understand the mechanisms regulating differential miRNA expression in depression and as predictors/mediators of antidepressant response. In particular, we need to understand the relationship between peripheral and brain miRNA levels and the extent to which we can use information from peripheral miRNA to infer levels of the same miRNA in the brain. One could be skeptical about this hypothetic correlation between brain and peripheral tissue and may believe a robust ‘blood–brain barrier’ exists in biomarker development in psychiatry; however, a

substantial evidence supports blood–brain correlations in miRNA levels of expression. First, brain and peripheral tissue such as blood share common biological processes (56), and miRNAs circulate in blood and other bodily fluids. They are stably transported in exosomes, double-lipid layered vesicles, which suggests that circulating miRNAs may act as molecular signals between different cells and tissues (21). Moreover, circulating miRNA levels could change with physiological changes or according to treatment (57-59). These characteristics provide support for miRNAs as potential biomarkers of disease, and—in the case of MDD—it is tempting to speculate that circulating miRNAs may reflect biological changes in the brain due to disease or antidepressant treatment effects. Finally, an innovative study (48) used neuro-imaging to demonstrate a correlation between miRNA levels of expression in the blood and brain activity. Although this study needs to be replicated in larger samples, it is worth noting that this blood–brain barrier could be lifted in the development of biomarkers. Moreover, animal studies could include blood sampling to systematically assess the actual correlation between both tissues for a given potential biomarker.

Given the massive burden of MDD worldwide, the development of biomarkers of antidepressant response could be considered urgent. The lines of evidence described in this review allow for hypotheses that miRNAs could be a part of the future of personalised psychiatry.

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Conflicts of interest

Raoul Belzeaux, Rixing Lin and Gustavo Turecki have no conflicts of interest.

Table 1: Steps in biomarker development

Step	Aim	Tool
Analytical validity	Level of detection; precision; repeatability	Detection range; coefficient of variation or intra-class correlation coefficient
Clinical validity	Accuracy or ability to predict a phenotype	Area under the curve from ROC curve; sensibility and specificity, likelihood ratio; odds ratio from logistic regression
Clinical utility	Ability to improve diagnosis or prognosis	Comparison with gold standard in randomized control trial
Clinical usefulness	Feasibility in current practice	Accessibility of the techniques, limitations due to sample processing and storage
Healthcare programme utility	Ability to reduce healthcare system costs	Evaluation of savings in medico-economic study
Biological plausibility	Functional analysis	Animal model, cellular model and other procedures to determine causal links between phenotype and biomarker

ROC: receiver operating characteristic

Table 2: Studies in which microRNA have been associated with major depression and/or antidepressant response with several lines of evidence

MicroRNA	Post-mortem brain studies in MDD	Peripheral study in MDD	Peripheral study in AD response	Animal study (brain)	Other evidence
miR-101b	(33)			(34)	
miR-107		(49)		(31)	
miR-1202	(46)	(46)	(46)	(46)	(46-48)
miR-218	(39)			(39)	
miR-124		(50)	(50)		
miR-124-3p	(38)	(38)		(38)	(38)
miR-125a				(31)	
miR-132				(31)	(27-29)
miR-135a	(45)	(45)	(45)	(45)	
miR-142-3p				(31)	
miR-145			(49)	(31)	
miR-16		(43)		(41-43)	
miR-200c		(49)		(31)	
miR-20b	(33)		(49)		
miR-24-3p		(37), (55)			
miR-34a	(33), (40)				
miR-381		(49)		(31)	
miR-425-3p		(37), (49)			
miR-494	(33)	(49)			

AD: antidepressant, MDD: major depressive disorder

Table 3: Human studies with the most robust microRNA associations with antidepressant response

MicroRNA	Patients (n)	Treatment, duration (tissue)	Treatment response criteria	Results	References
miR-1202	32	Citalopram, 8 weeks (whole blood)	Remission	Lower expression was predictive for remission; increase in expression level correlated with clinical improvement	(46)
miR-124	32	Personalized antidepressant, 8 weeks (PBMC)	Response	Decrease in expression level after treatment in responders; correlation between change in expression level and clinical improvement	(50)
miR-135a	24	CBT, 12 weeks, or escitalopram, 12 weeks (whole blood)	None	Trend for higher expression after CBT vs. escitalopram ($p=0.08$ in two-way ANOVA)	(45)
miR-145	9	Personalized antidepressant, 8 weeks (PBMC)	Response (only responders included)	Increase in expression level during treatment in responders	(49)
miR-20b	9	Personalized antidepressant, 8 weeks (PBMC)	Response (only responders included)	Increase in expression level during treatment in responders	(49)

ANOVA: analysis of variance, CBT: cognitive behavioural therapy; PBMC: peripheral blood mononuclear cells

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