genes

MDPI

Article

Differential Expression of tRNA-Derived Small RNA Markers of Antidepressant Response and Functional Forecast of Duloxetine in MDD Patients

Xiaoyan Wang [1,†], Ming Gao [2,†], Jing Song [3], Miaolong Li [4], Yu Chen [5], Yingfang Lv [1], Wei Jia [6] and Bingbing Wan [1,*]

[1] Key Laboratory of Systems Biomedicine (Ministry of Education), Shanghai Center for Systems Biomedicine, Shanghai Jiao Tong University, Shanghai 200240, China; cathywxy@sjtu.edu.cn (X.W.)
[2] School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[3] School of Medicine, Renji Hospital, Shanghai Jiao Tong University, Shanghai 200240, China
[4] School of Chemical Science and Engineering, Tongji University, Shanghai 200070, China
[5] Department of Clinical Medicine, He University, Shenyang 110163, China
[6] Department of Pharmacology and Pharmacy, University of Hong Kong, Hong Kong, China
* Correspondence: wanb@sjtu.edu.cn
† These authors contributed equally to this work.

Abstract: Background/Objectives: Duloxetine, despite being a leading treatment option for major depressive disorder (MDD), exhibits a relatively low adequate response rate when used as a monotherapy, and the fundamental molecular mechanisms remain largely elusive. tRNA-derived small RNA (tsRNA) is a particularly interesting and new class of molecules that is becoming increasingly noticeable for investigation. Methods: We integrated small RNA sequencing with bioinformatics approaches to dissect the expression profiles of tsRNAs and decipher their functional roles post-duloxetine treatment. Subsequently, molecular docking experiments were carried out to validate the potential functions. Results: Ten tsRNAs significantly changed in the duloxetine response group after an 8-week therapy. Correlation analyses revealed that these tsRNAs predominantly interacted with miRNAs across multiple biological pathways and processes, such as the ECM-receptor interaction and B cell activation. Molecular docking analysis corroborated the binding capabilities of duloxetine with key proteins associated with ECM1 and BAFF, respectively. Conclusions: The identified changes in tsRNAs can precisely mirror the response of duloxetine in MDD treatment, offering novel insights into the underlying mechanisms of duloxetine action.

Keywords: duloxetine; functional prediction; small non-coding RNAs; tRNA-derived small RNAs; biomarkers; ECM1; BAFF

check for updates

Academic Editor: Antonio Drago

Received: 30 November 2024
Revised: 14 January 2025
Accepted: 23 January 2025
Published: 27 January 2025

Citation: Wang, X.; Gao, M.; Song, J.; Li, M.; Chen, Y.; Lv, Y.; Jia, W.; Wan, B. Differential Expression of tRNA-Derived Small RNA Markers of Antidepressant Response and Functional Forecast of Duloxetine in MDD Patients. *Genes* **2025**, *16*, 162. https://doi.org/10.3390/genes16020162

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Major depression represents a heterogeneous ailment, characterized by a multiplicity of symptoms, including cognitive impairments and diverse forms of physical disabilities. This condition imposes significant health and social burdens on a global scale, as attested by numerous studies [1]. Among the array of treatment modalities available, such as psychological behavior intervention and diet and nutrition intervention, drug therapy remains the cornerstone of treatment. Antidepressants have demonstrated pronounced efficacy in treating moderate to severe depressive episodes; nevertheless, the individual responses to these pharmacological interventions exhibit substantial variability. Despite

the wide range of antidepressants in clinical use today, a staggering 30–40% of patients fail to achieve a full therapeutic response [2,3]. The lack of response not only prolongs patients' suffering but also places an onerous burden on both patients and their families. Thus, delving deeper into the factors that influence antidepressant treatment outcomes is of paramount importance and urgency.

Duloxetine, being one of the most prevalently prescribed antidepressants, has drawn considerable clinical attention regarding its therapeutic efficacy and has been the subject of in-depth investigations [4,5]. Gene sequencing studies have revealed genetic variants upstream of STAC1 that are correlated with the treatment response in patients with depression, whether they were administered duloxetine or a placebo [6]. Non-coding RNAs (ncRNAs), a category of RNA molecules that do not participate in protein translation, function as regulatory elements, akin to molecular spinners and switches, modulating the transcriptional activity of gene expression. Dysregulation of ncRNAs frequently disrupts the biochemical pathways implicated in major depressive disorder (MDD) [7,8]. The identification of changes in ncRNAs in response to drugs used for treating MDD holds the promise of uncovering biomarkers capable of predicting drug responses, thereby facilitating the efficient screening of patients who exhibit minimal or no response to traditional medications [9].

Transfer RNAs (tRNAs), a class of ncRNAs that rank second in abundance within cells, can be enzymatically cleaved into a diverse assortment of ncRNA fragments, typically ranging from 18 to 40 nucleotides in length. These fragments, generated from either precursor tRNAs or mature tRNAs through the action of specific endonucleases, have recently emerged as functionally significant small non-coding RNAs, termed tsRNAs. Mounting evidence implicates multiple tsRNA dysregulations in various human diseases [10–12]. Notably, tsRNAs have been reported to modulate pathophysiological alterations in neurological disorders, such as neurodegeneration and nerve injury, suggesting their potential role in regulating mental disorders [13]. Current research indicates that specific tRNA-modifying enzymes and tsRNAs could serve as promising diagnostic biomarkers and therapeutic targets [14]. However, to date, no studies have explored the role of tsRNAs in human depression.

In the present study, we sought to investigate tsRNAs as prospective biomarkers for antidepressant response by employing small-RNA sequencing on matched specimens from patients with MDD. These patients were participants in a placebo-controlled, randomized trial evaluating duloxetine treatment, with samples collected both prior to treatment initiation and eight weeks after the commencement of therapy. Our findings demonstrate that the levels of ten tsRNAs are differentially regulated in relation to antidepressant response and are involved in modulating genes associated with crucial biological processes, including extracellular matrix (ECM)-receptor interaction, the transforming growth factor-β (TGF-β) signaling pathway, fatty acid biosynthesis, thyroid hormone synthesis, the Hippo signaling pathway, the plasma membrane signaling receptor complex, and humoral immune response. Further molecular docking analysis confirmed the binding potential of duloxetine and key proteins, verifying the pathways involved in the drug response of duloxetine involved in the above tsRNA.

2. Materials and Methods

Dataset: The miRNA-seq sequencing and miRNA expression files were collected from GSE97154 [15]. This study is a double-blind clinical trial that is registered at www.ClinicalTrials.gov (11984A NCT00635219). A total of 258 patients (males n = 80; females n = 178) were enrolled and diagnosed with MDD. Participants were randomly designated to obtain either a placebo or 60 mg of duloxetine. Peripheral blood samples

were collected at the beginning of the study and following the treatment period. For inclusion criteria, patients were aged from 19 to 74 and were diagnosed with MDD and a major depressive episode (MDE) lasting more than three months, having a severity score on the Montgomery–Asberg Depression Rating Scale at baseline of no less than 22. For exclusion criteria, patients had undergone at least two prior antidepressant (AD) treatments, experienced electroconvulsive therapy within the six weeks preceding the study or had a major depressive episode (MDE) along with bipolar disorder, psychotic features, or a recent substance use disorder. The percentage change of Montgomery–Asberg Depression Rating Scale (MADRS) scores was calculated (from week 0 to week 8 therapy) to quantify the therapy response. The responder/non-responder were categorized according to a great decrease in Montgomery–Asberg Depression Rating Scale scores from week 0.

Sequencing data analysis: All sequencing data were sequenced on the HiSeq2500 Illumina sequencer (Illumina, San Diego, CA, USA). The Cutadpt 2.1 was utilized, and low-quality reads were filtered [16]. The expression data of tsRNAs were obtained after clean reads were aligned to the mature-tRNA genome using MINTmap(v2.0) [17]. Missing values were imputed by MetImp 1.2 [18].

Statistical analysis: We conducted a comparison of treated patients (week 8) and baseline (week 0) using the student *t*-test. Correlation analysis between the single vectors was performed using the Spearman correlation. For two matrix correlation analysis, the Spearman correlation first analyzed the correlation analysis between the single tsRNA with single miRNA, and then miRNAs with $p < 0.001$ were selected for the matrix Mantel test. DIANA tools were used for miRNA pathway analysis [19]. R software (version 4.0.2) was employed for all analyses.

Target Prediction: The RNAhybrid algorithm was employed to forecast the potential binding mRNAs' targets, using a screening criteria of energy <−25 kcal/mol) (https://bibiserv.cebitec.uni-bielefeld.de/, accessed on 28 January 2024). Shingo was applied to analyze cellular components and biological processes, as well as identify potential functions (http://bioinformatics.sdstate.edu/go/, accessed on 28 January 2024).

Molecular docking: The crystal structure of the key protein (ECM1 and BAFF) was obtained in the Protein Data Bank (PDB, https://www.rcsb.org/), respectively. The 3D structures of duloxetine were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The Autodock 4.0 was applied to perform molecular docking and calculate binding affinity. Each calculation generated 50 structures, and the molecular docking output was prioritized according to the frequency of possible ligand-binding sites and free-energy score. The docking results of ECM1 proteins and duloxetine were visualized by PyMOL 2.2.0 software.

3. Results

3.1. Differential tsRNA Expression After Duloxetine Therapy

3.1.1. The Workflow of the Study

The study began by obtaining sequencing data in fastq format from the GSE97154 dataset. The sequencing reads were then processed to remove adapters, ensuring high-quality data for subsequent analysis. The workflow of the study is shown in Figure 1.

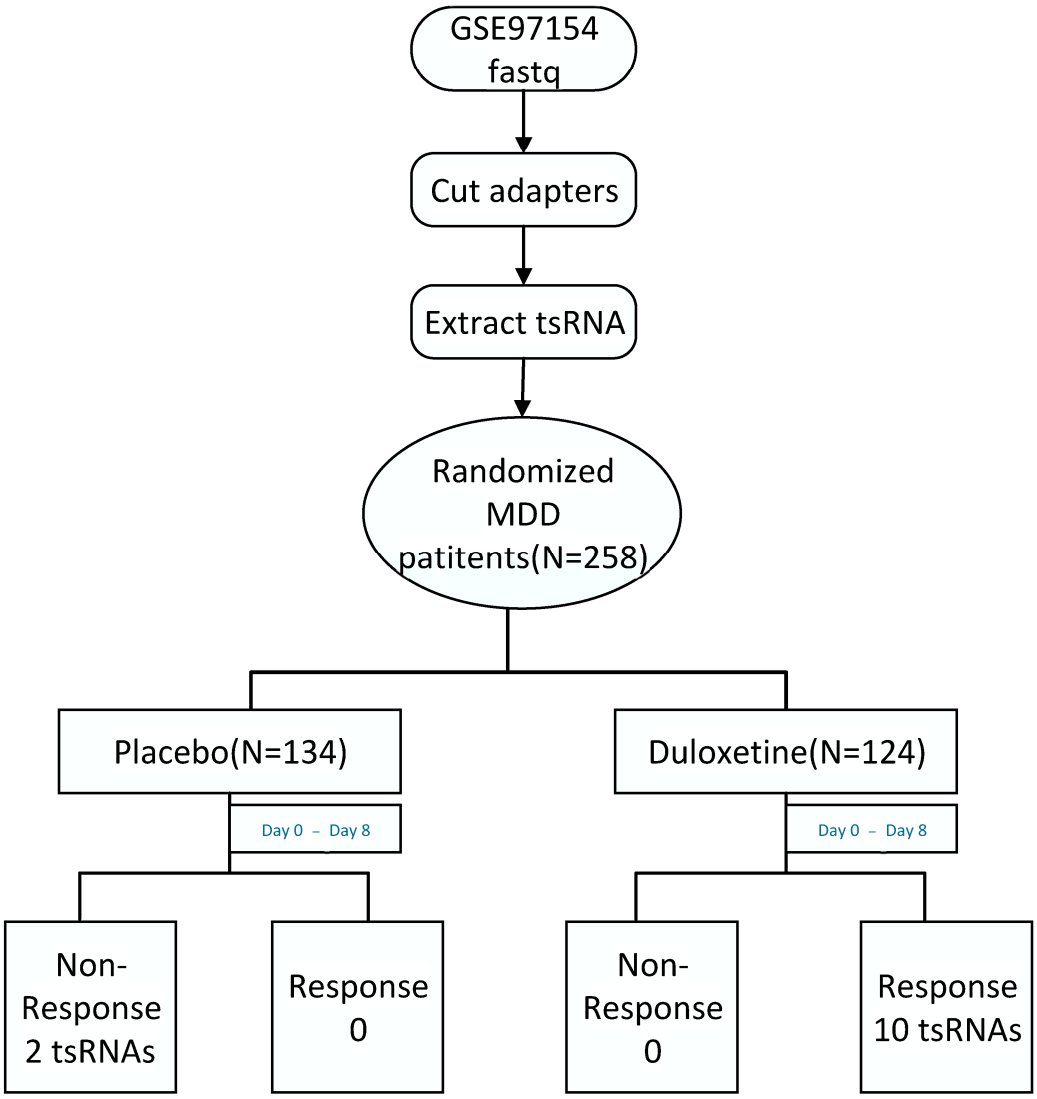

Figure 1. Workflow of data process.

3.1.2. Differential tsRNA Expression Identification

From the processed data, transfer RNA-derived small RNAs (tsRNAs) were extracted for further investigation. After we extracted the tsRNA expression data from small-RNA-sequencing blood samples, we compared the expression of tsRNAs after and before therapy (week 8 to week 0). A 5% false discovery rate (FDR) using the Benjamini–Hochberg correction for multiple testing was applied in differential analysis. The findings indicate a differential expression of ten tsRNAs in the duloxetine response group after an 8-week treatment period (Table 1) and two tsRNAs in the placebo non-responsive group (Table 2). We analyzed each group's overlap through the Venn diagram and found that tRF-36-D4ZWRNU3KQ9MV1B overlapped in the duloxetine response and placebo non-responsive groups (Figure 2). We further analyzed the expression of ten tsRNAs significantly changed in the duloxetine response group before and after treatment and found that ten tsRNAs cluster into two principal classes in the heatmap (Figure 2). These tsRNAs with p value < 0.05 were calculated in a paired student t-test and listed (Supplementary Table S1).

Table 1. Duloxetine response Signiant tsRNAs (FDR < 0.001).

Name	Sequence	*p* Values	Fold Change	FDR
tRF-20-9LON4VN1	TGGTAGAATTCTCGCCTGCC	2.65×10^{-9}	0.567917175	1.08×10^{-6}
tRF-31-PNR8YP9LON4VD	GCATTGGTGGTTCAGTGGTAGAA TTCTCGCC	2.02×10^{-8}	0.655811181	2.73×10^{-6}
tRF-19-VBY9PY11	TAGAATTCTCGCCTGCCAC	1.75×10^{-8}	0.542665077	2.73×10^{-6}
tRF-50-PNR8YP9LON4VN1EH6KK8	GCATTGGTGGTTCAGTGGTAGAA TTCTCGCCTGCCACGCGGGAGGC CCGG	4.47×10^{-8}	0.684414445	4.53×10^{-6}
tRF-32-PNR8YP9LON4V3	GCATTGGTGGTTCAGTGGTA GAATTCTCGCCT	2.62×10^{-6}	0.744936648	0.000178
tRF-20-WB8689SV	TCGAATCCCATCCTCGTCGC	2.98×10^{-6}	1.259833712	0.000178
tRF-36-D4ZWRNU3KQ9MV1B	AAGTGTTTGTGGGTTTAAGTCCC ATTGGTCTAGCCA	3.08×10^{-6}	1.489983712	0.000178
tRF-20-VBY9PYKH	TAGAATTCTCGCCTGCCACG	4.19×10^{-6}	0.705437268	0.000213
tRF-33-86V8WPMN1E8Y0E	TCCCATATGGTCTAGCGGTTAGG ATTCCTGGTT	2.02×10^{-5}	1.319922659	0.00082
tRF-43-7673FEWS3V2VR0PSDZ	GTTCAGTGGTAGAATTCTCGCCT GCCACGCGGGAGGCCCGGGT	1.85×10^{-5}	1.493597202	0.00082

Table 2. Placebo non-response Signiant tsRNAs (FDR < 0.001).

Name	Sequence	*p* Values	Fold Change	FDR
tRF-34-10I9BZBZOS4YE2	AGGAGATTTCAACTTAACTTGAC CGCTCTGACCA	1.82×10^{-7}	1.617784657	7.38×10^{-5}
tRF-36-D4ZWRNU3KQ9MV1B	AAGTGTTTGTGGGTTTAAGTCCC ATTGGTCTAGCCA	5.62×10^{-7}	1.697292931	0.000114

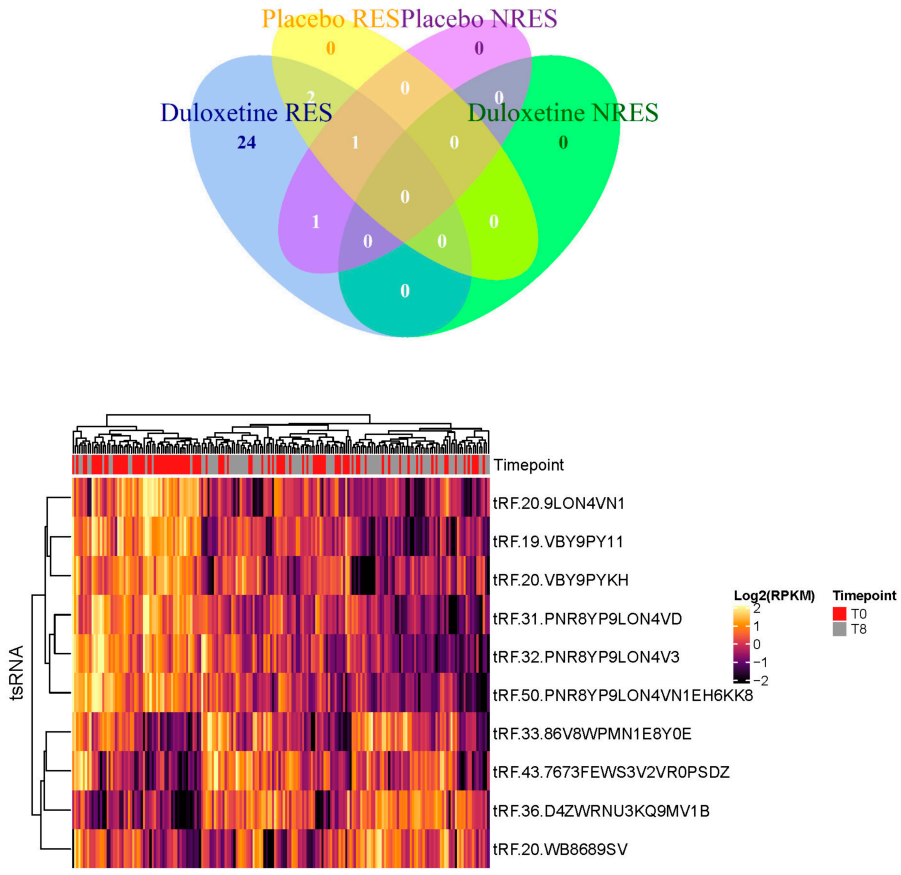

Figure 2. Venn diagram of each group and duloxetine treatment response tsRNA expression analysis. Unsupervised hierarchical clustering of all significant tsRNA markers for duloxetine treatment response. Each row is tsRNA, and the column is the patient sample.

3.2. Correlation Analysis and Functional Enrichment Analysis

3.2.1. Correlation Analysis of tsRNAs and tsRNA Expression

To analyze the relationship between these tsRNAs, we applied Spearman correlation analysis. The heatmap in Figure 3 shows that these tsRNAs are divided into two categories, each of which is positively correlated internally. Following an 8-week treatment, we observed that among the two types of tsRNA, four were upregulated, and six were downregulated (box plots in Figure 3). Unlike miRNAs that are widely studied, only a few tsRNA functions are known. Here, we use the correlation analysis of tsRNAs and miRNAs, generated by previous research, and enhance the functions of these miRNAs, which are strongly related to tsRNAs, to predict the functions attributed to them.

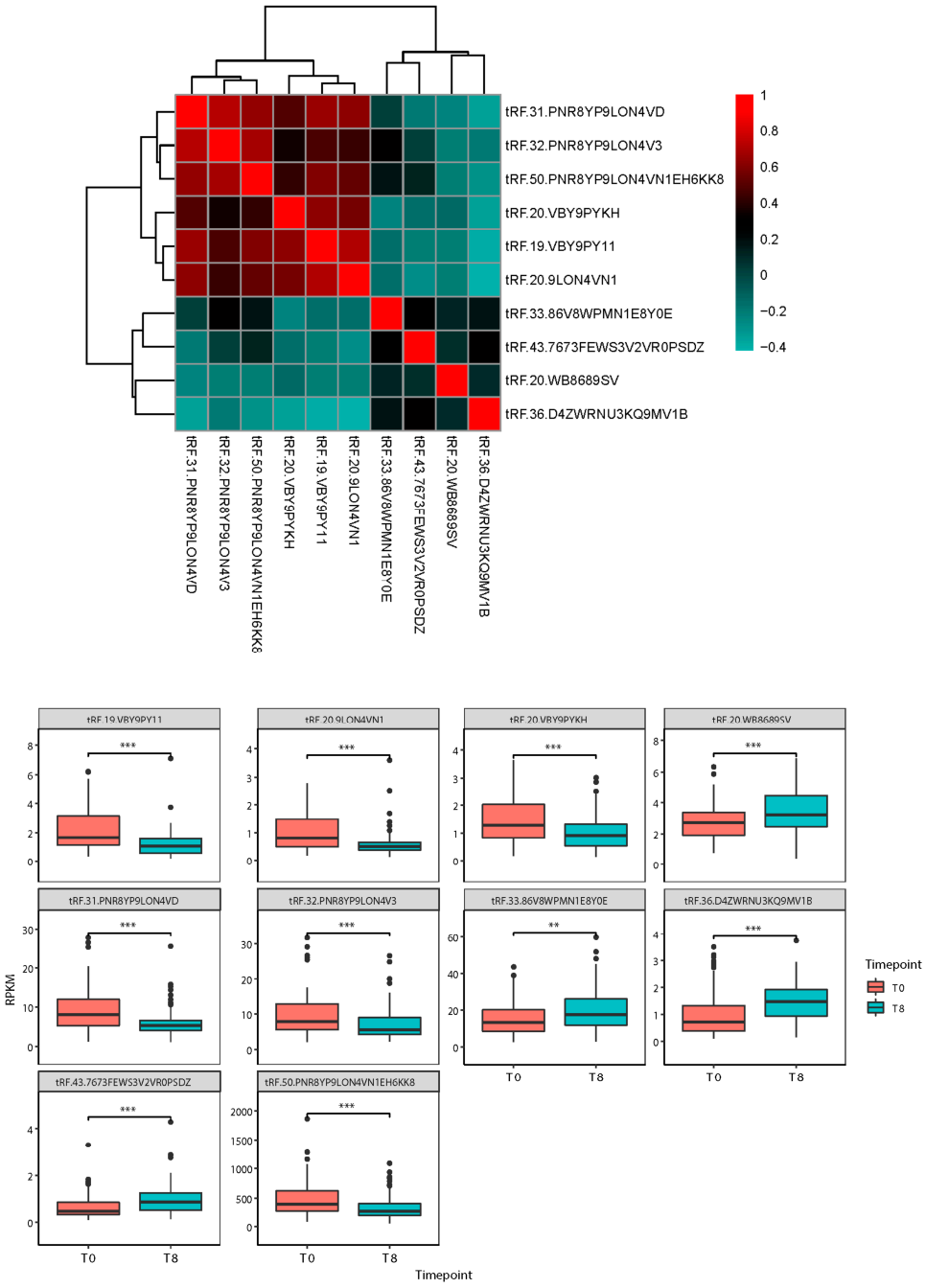

Figure 3. Correlation analysis of tsRNAs and tsRNA expression data. (** p value less than 0.01, *** p value less than 0.001).

3.2.2. Correlation Analysis Between Significant tsRNAs and miRNAs

Figure 4A illustrates the correlation analysis between tsRNAs and miRNAs, highlighting specific interactions that could play crucial roles in regulating the molecular pathways implicated in MDD. This figure reveals patterns of connectivity that suggest both direct and indirect regulatory relationships, affecting the transcriptional landscape in response to duloxetine treatment. Particularly, we can observe that that tRF.50.PNR8YPLON4VN1EH6KK8 and tRF.33.86V8WPMN1E8Y0E have more interaction pathways with miRNAs, indicating their potentially influential roles in regulating or being regulated by the expression of various miRNAs.

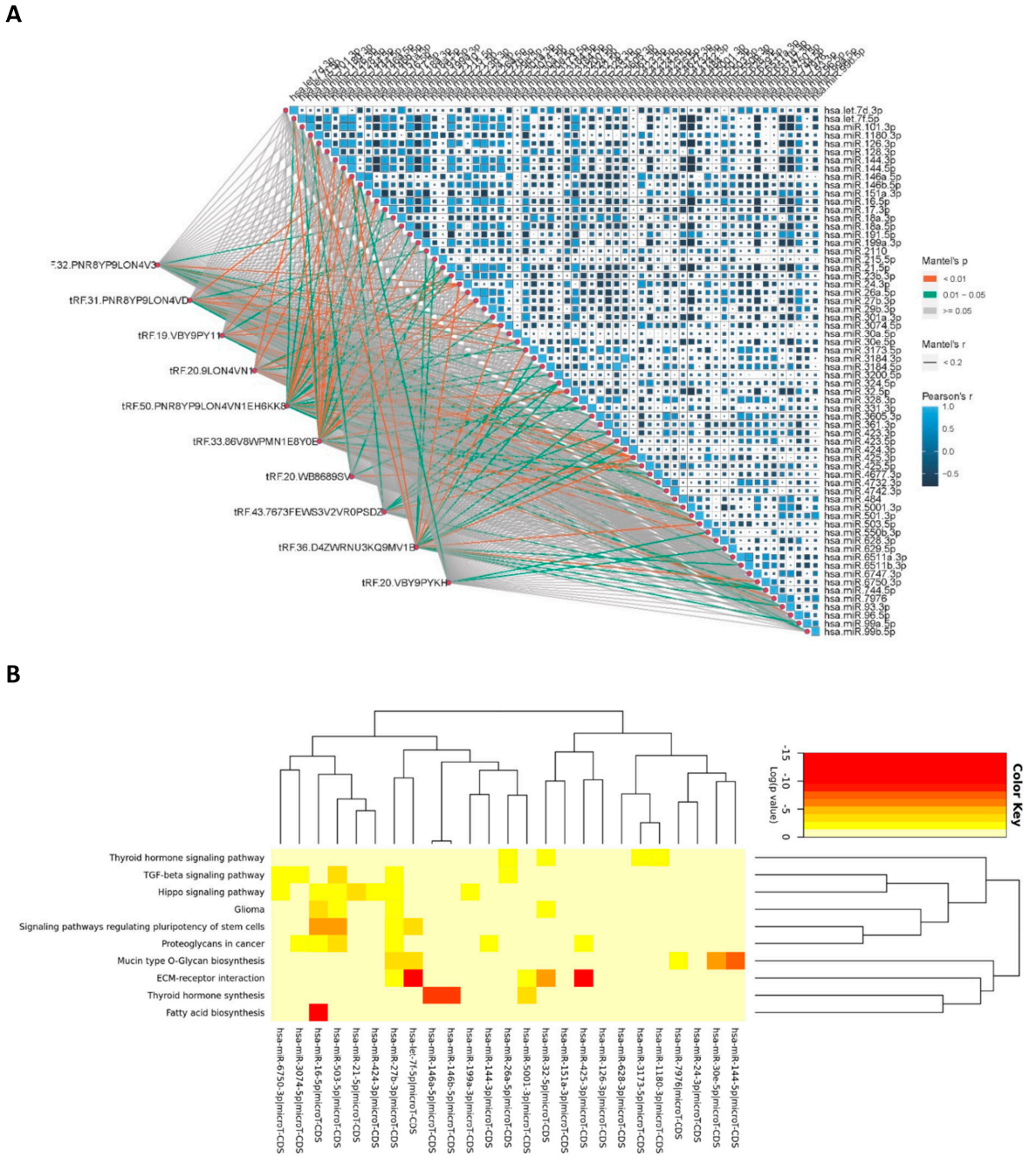

Figure 4. Correlation analysis between significant tsRNAs and miRNAs (**A**) and correlation analysis miRNA function study (**B**).

3.2.3. Correlation Analysis miRNA Function Study

The results of the correlated miRNA function study are shown in Figure 4B. miR-146a and miR-146b were correlated with thyroid hormone synthesis. The ECM-receptor interaction was correlated with miR-425, while fatty acid biosynthesis was correlated with miR-16, respectively.

We selected miRNAs with Mantel's *p* value less than 0.01 for enrichment analysis. Enrichment analysis revealed that these miRNAs mainly interact with ECM-receptor interaction together with fatty acid biosynthesis, thyroid hormone synthesis, TGF-β, and the Hippo signaling pathway (Supplementary Table S2).

3.3. Bioinformatic Prediction of the Ten Significantly Expressed tsRNAs

The function of ten significantly expressed tsRNAs in the duloxetine response group was studied using bioinformatic techniques. Figure 5A depicts the enrichment analysis of the biological process. Among them, the notable enrichment and the significant terms discovered were, respectively, the plasma membrane signaling receptor complex and the humoral immune response in molecular function. The cellular component of tsRNA target genes is shown in Figure 5B with the identical findings.

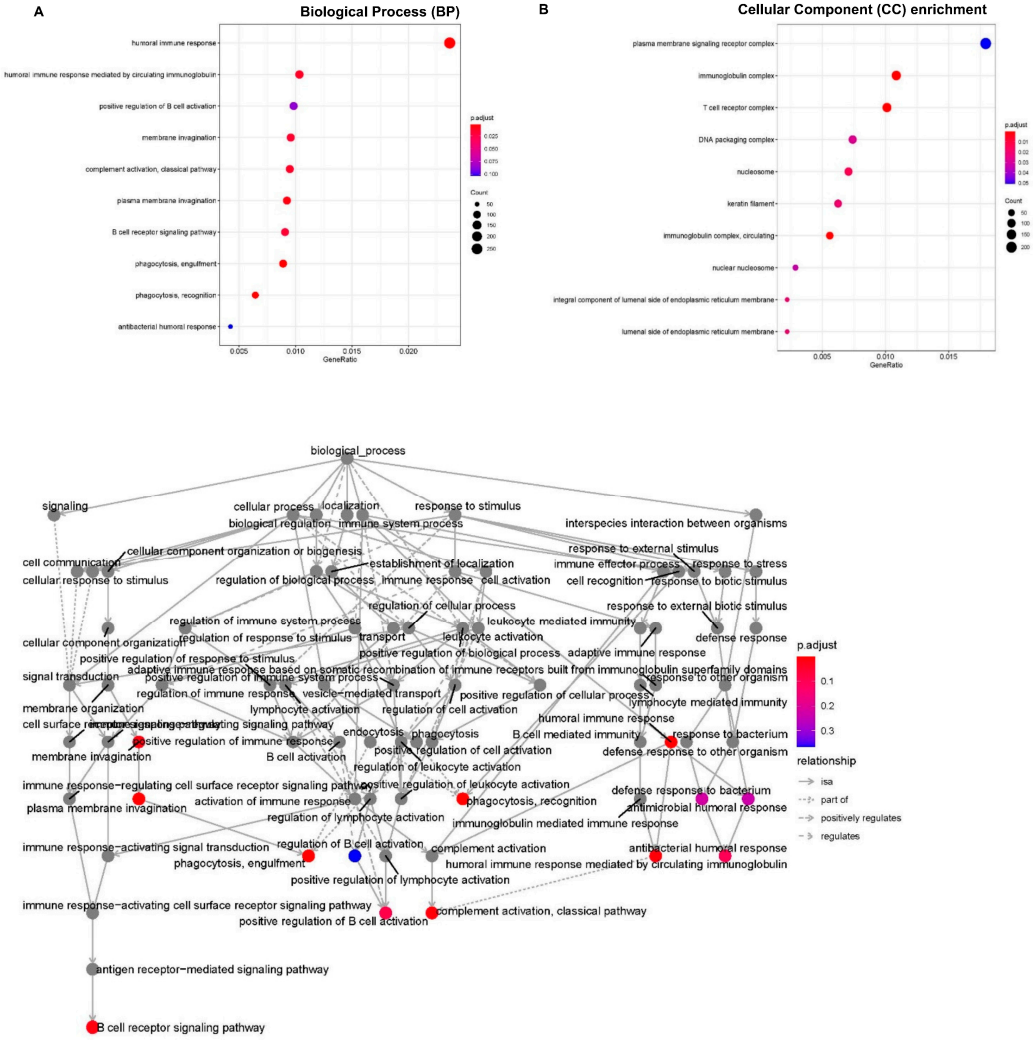

Figure 5. Biological process enrichment analysis and cellular component enrichment of tsRNA target genes. (**A**) The top 10 biological processes are presented in the bubble chart and in the whole network. (**B**) The top 10 cellular component enrichments are presented in the bubble chart (The area of the circle indicates the tsRNA target gene number).

3.4. Duloxetine with Its Corresponding Proteins

According to the result of functional enrichment analysis, ECM1, an important protein in ECM-receptor interaction, was proposed to find a potential relationship with duloxetine. Molecular docking analysis displayed the possibility that duloxetine binds to GLU199 of ECM1. The predicted binding energy is −4.84 kcal/mol (Figure 6A), which indicates that there is a strong affinity between protein and ligand. By observing the protein surface model, one can also see that the ligand is only attached to the protein surface, and a hydrogen bond is formed between the ligand and the protein residue, which fully demonstrates the interaction between duloxetine and the ECM1 protein.

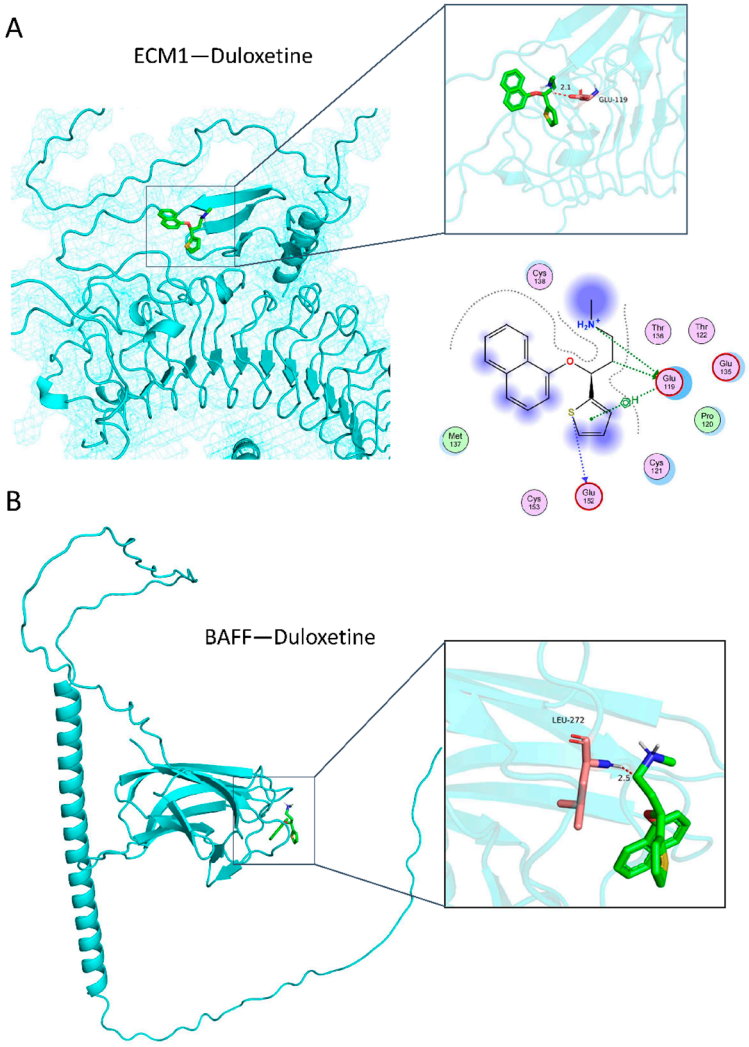

Figure 6. The 3D docking conformations of ECM1 and duloxetine (**A**); BAFF and duloxetine (**B**).

According to biological process enrichment analysis and cellular component enrichment of tsRNA target genes (Figure 5), B cell activation is the most frequent biological process. Hence, BAFF (B cell activating factor) is selected. Our docking results show that the interaction free energy between BAFF and duloxetine is −4.44 kcal/mol, which indicates that there is a strong affinity between protein and ligand. By observing the protein surface model, we found that small molecules stick to the protein surface and zoom in on the area. We found that the structure formed on the protein surface is similar to that of small molecules, which is conducive to inducing small molecules to bind to it. At the same time, we also showed the unit structure between protein and ligand and found

that hydrogen bonds are formed between leucine at position 272 and the ligand, further stabilizing the complex.

4. Discussion

Duloxetine, a prominent member of the class of selective serotonin and norepinephrine reuptake inhibitors (SNRIs), has carved out a significant niche in clinical practice for the management of major depressive disorder (MDD) [20,21], whereas SSRIs, like Fluoxetine, Paroxetine, and Sertraline, primarily act on the reuptake of 5-HT. Our choice was based on duloxetine's dual serotonin-norepinephrine reuptake inhibition mechanism and its established clinical efficacy in treating major depressive disorder (MDD) [21]. It was also demonstrated to possess neuroprotective effects, likely via these pathways, in addition to its capacity to modify neurotransmitter signaling [5]. At cellular level, emerging evidence reports that alterations in autophagy, inflammation, coke death, and apoptosis pathways can potentially influence its development and progression [22]. Given this intricate web of cellular events and their far-reaching implications, it becomes eminently pertinent to explore the possibility of identifying reliable biomarkers.

The employment of miRNA biomarkers to monitor patient responses in mood disorders marks a transformative leap in treatment paradigms. Multiple studies have indicated that interventions like SNRIs, SSRIs, serotonin modulation, and electroconvulsive therapy (ECT) achieve their therapeutic impacts, in part, by targeting miRNAs [23], clearly suggesting miRNAs' potential as powerful indicators for gauging treatment efficacy.

In mice with CUMS-induced depression, miR-134 and miR-124a were markedly elevated in the frontal lobe and hippocampus, but these levels declined post-duloxetine treatment [24,25]. Clinically, there are discernible differences in miRNA expression between patients who respond to duloxetine and those who do not. Here, miR-16, miR-146a, and miR-21p have been identified as promising markers linked to remission under duloxetine treatment [15]. On the other hand, multiple miRNAs have been linked to either a response to treatment or a heightened risk of major depression. These significant findings were validated that downregulated miR-146a, miR-24, miR-425, and miR-3074 after treatment were strongly correlated, indicating a common mode of action [15].

By delving into miRNA profiles, we meticulously examined the alterations in miRNA expression triggered by duloxetine treatment. Our findings strongly suggest that duloxetine's therapeutic function may well be intertwined with its influence on miRNA expression patterns, thereby affirming its role as a modulator of miRNA levels, which aligns with existing literature [26].

Enrichment analysis unveiled that tsRNA-related miRNAs partake in critical biological processes such as ECM-receptor interaction, thyroid hormone synthesis, fatty acid biosynthesis, the TGF-β signaling pathway, and the Hippo signaling pathway. Meanwhile, extensive research has explored the connections between fatty acid biosynthesis, the TGF-β signaling pathway [27], thyroid hormone synthesis, and depression [28].

Investigations have spotlighted the fact that the extracellular matrix (ECM) serves as a vital conduit for communication, potentially influencing behavior stress regulation and depression [29,30], with the intertwined connection between the ECM and immune processes [31,32]. The ECM has also been definitively shown to hold a crucial role in orchestrating inflammatory and neuropathic pain, as corroborated by multiple studies [33–35]. Widely acknowledged in the medical field, duloxetine exhibits remarkable efficacy not only in alleviating the depressive symptoms of patients with major depressive disorder (MDD) but also in substantially ameliorating diverse forms of pain [36,37]. However, the precise mechanism underlying its pain-relieving capabilities remains elusive. Our molecular docking experiments yielded a clear and potent indication of duloxetine's interaction

with ECM1, a key protein within the ECM framework (Figure 6A). This discovery not only provides robust support for earlier enrichment analysis outcomes but also posits that the therapeutic impact of duloxetine on MDD symptoms could be intertwined with its binding to the ECM. This binding, potentially implicating immune-related pathways, offers tantalizing clues about the drug's latent mechanisms for pain regulation.

Simultaneously, we carried out an in-depth analysis of the tsRNA-miRNA function concerning duloxetine's effects. Pathway enrichment analysis highlighted the plasma membrane signaling receptor complex and the humoral immune response as the most prominent terms in molecular function and biological processes. Among them, the B cell activation pathway emerges as a focal point of interest. In light of this, BAFF (B cell activating factor), a cytokine that plays a pivotal role in activating B cells, belonging to the tumor necrosis factor (TNF) ligand family, was chosen for molecular docking experiments with duloxetine. The outcomes were quite revealing, demonstrating a notable binding affinity between the two entities (Figure 6B). BAFF is closely related to autoimmunity and immune regulation [38,39], thereby making its interaction with duloxetine a potentially crucial aspect in understanding the drug's immunomodulatory potential and therapeutic implications.

Given the findings regarding interactions with BAFF, ECM1, and TGF-β, it would be relevant to discuss the role of neuroinflammation in depression. Duloxetine was reported to possess anti-inflammatory (decreasing TGF-β proteins) and antioxidant properties to regulate the expression of angiogenesis and neurotrophic factors [40], which may relate to neuroinflammation and the broader immunological mechanisms underlying depression. This will tie in with emerging evidence of SSRIs exhibiting properties beyond serotonin reuptake inhibition, which could provide a more holistic understanding of their therapeutic effects.

tRF-36D4ZWRNU3KQ9MV1B was retained in the analysis, and its significance was interpreted within the broader context of depression and antidepressant mechanisms. This overlap does not diminish its importance but rather underscores the complexity of tsRNA-mediated regulation in MDD. Further experimental validation is suggested to unravel its precise functional role in these overlapping conditions.

Actually, one tsRNA has been proven to serve as a key target in depression, and the silencing of it diminishes the occurrence of ferroptosis and safeguards neurons from injury [41]. Significant downregulation of tsRNA was evident after an 8-week treatment course and functioned as a promising baseline predictor of a patient's response to antidepressant therapy [42]. Consistently, our results also suggest that tsRNA serves as a predictive biomarker for the drug treatment effect of major depressive disorder, indicating that specific tsRNAs present in peripheral blood show a significant response to depressive disorders and their symptoms, and the underlying mechanism is worthy of in-depth exploration.

The limitation of this study lies in the fact that it only explored data from public databases without performing multi-center validation. The sequencing results need to be further verified in other samples from multiple clinical centers in the future. Moreover, the results of the molecular docking also demand further molecular biological experimental validation to confirm that the drug duloxetine has the actual ability to bind to and even regulate the receptor target. Although this study has completed the expression profile and functional prediction, there remain questions that require further exploration. Additionally, experimental verification both in vitro and in vivo is needed to identify the functions of the candidate tsRNAs and also the related signaling pathways mentioned above. This will provide valuable guidance in elucidating the antidepressant mechanism and even in developing new indications of duloxetine.

5. Conclusions

Our study has successfully demonstrated that the alterations in tsRNA expression patterns can accurately reflect the response of duloxetine in MDD treatment. This not only provides novel insights into the long—elusive molecular mechanisms of duloxetine action but also paves the way for developing more accurate diagnostic and prognostic tools.Moreover, the molecular docking analysis validating duloxetine's binding with key proteins related to ECM1 and BAFF enriches our understanding of its therapeutic mechanism. These findings are expected to stimulate further research on the complex interactions between tsRNAs and other cellular components. Such research may lead to innovative therapeutic strategies, enhancing the effectiveness of duloxetine or other antidepressants, thus potentially alleviating the burden of MDD.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/genes16020162/s1: Table S1: A list of tsRNAs that *p*-value less than 0.05 when the paired student *t*-test was evaluated; Table S2: Pathway analysis of ten tsRNAs predicted target mRNAs.

Author Contributions: Conceptualization, B.W. and X.W.; methodology, X.W.; software, M.G.; validation, J.S. and M.L.; formal analysis, Y.C.; investigation, Y.L.; resources, J.S. and M.G.; data curation, X.W.; writing—original draft preparation, X.W. and M.G.; writing—review and editing, W.J.; visualization, M.L.; supervision, B.W.; project administration, X.W.; funding acquisition, B.W. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program of China (Grant no. 2022YFC2407003) and the Interdisciplinary Program of Shanghai Jiao Tong University (YG2022QN015). Science and Technology Commission of Shanghai Municipality, China (22ZR1432700 to B. Wan).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Santomauro, D.F.; Vos, T.; Whiteford, H.A.; Chisholm, D.; Saxena, S.; Ferrari, A.J. Service coverage for major depressive disorder: Estimated rates of minimally adequate treatment for 204 countries and territories in 2021. *Lancet Psychiatry* **2024**, *11*, 1012–1021. [CrossRef]
2. Cipriani, A.; Furukawa, T.A.; Salanti, G.; Chaimani, A.; Atkinson, L.Z.; Ogawa, Y.; Leucht, S.; Ruhe, H.G.; Turner, E.H.; Higgins, J.P.T.; et al. Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: A systematic review and network meta-analysis. *Lancet* **2018**, *391*, 1357–1366. [CrossRef]
3. Cui, L.; Li, S.; Wang, S.; Wu, X.; Liu, Y.; Yu, W.; Wang, Y.; Tang, Y.; Xia, M.; Li, B. Major depressive disorder: Hypothesis, mechanism, prevention and treatment. *Signal Transduct Target Ther.* **2024**, *9*, 30. [CrossRef] [PubMed]
4. Breilmann, J.; Furukawa, T.A.; Becker, T.; Koesters, M. Differences in the placebo response in duloxetine and venlafaxine trials. *Acta Psychiatr. Scand.* **2018**, *137*, 472–480. [CrossRef]
5. Dhaliwal, J.S.; Spurling, B.C.; Molla, M. *Duloxetine*; StatPearls: Treasure Island, FL, USA, 2024.
6. Maciukiewicz, M.; Marshe, V.S.; Tiwari, A.K.; Fonseka, T.M.; Freeman, N.; Kennedy, J.L.; Rotzinger, S.; Foster, J.A.; Kennedy, S.H.; Muller, D.J. Genome-wide association studies of placebo and duloxetine response in major depressive disorder. *Pharmacogenom. J.* **2018**, *18*, 406–412. [CrossRef] [PubMed]
7. Fakhri, S.; Darvish, E.; Narimani, F.; Moradi, S.Z.; Abbaszadeh, F.; Khan, H. The regulatory role of non-coding RNAs and their interactions with phytochemicals in neurodegenerative diseases: A systematic review. *Brief. Funct. Genom.* **2023**, *22*, 143–160. [CrossRef] [PubMed]

8. Prodan-Barbulescu, C.; Seclaman, E.P.; Enatescu, V.; Faur, I.F.; Ghenciu, L.A.; Tutac, P.; Pasca, P.; Grigorita, L.O. Evaluating the Connection between MicroRNAs and Long Non-Coding RNAs for the Establishment of the Major Depressive Disorder Diagnosis. *Biomedicines* **2024**, *12*, 516. [CrossRef]
9. Gibbons, A.; Sundram, S.; Dean, B. Changes in Non-Coding RNA in Depression and Bipolar Disorder: Can They Be Used as Diagnostic or Theranostic Biomarkers? *Noncoding RNA* **2020**, *6*, 33. [CrossRef]
10. Zhang, L.; Liu, J.; Hou, Y. Classification, function, and advances in tsRNA in non-neoplastic diseases. *Cell Death Dis.* **2023**, *14*, 748. [CrossRef]
11. Zhang, Q.; Zhao, X.; Sun, M.; Dong, D. Novel insights into transfer RNA-derived small RNA (tsRNA) in cardio-metabolic diseases. *Life Sci.* **2024**, *341*, 122475. [CrossRef]
12. Zhu, L.; Liu, X.; Pu, W.; Peng, Y. tRNA-derived small non-coding RNAs in human disease. *Cancer Lett.* **2018**, *419*, 1–7. [CrossRef]
13. Qin, C.; Xu, P.P.; Zhang, X.; Zhang, C.; Liu, C.B.; Yang, D.G.; Gao, F.; Yang, M.L.; Du, L.J.; Li, J.J. Pathological significance of tRNA-derived small RNAs in neurological disorders. *Neural Regen. Res.* **2020**, *15*, 212–221. [CrossRef] [PubMed]
14. Wu, D.; Li, X.; Khan, F.A.; Yuan, C.; Pandupuspitasari, N.S.; Huang, C.; Sun, F.; Guan, K. tRNA modifications and tRNA-derived small RNAs: New insights of tRNA in human disease. *Cell Biol. Toxicol.* **2024**, *40*, 76. [CrossRef] [PubMed]
15. Lopez, J.P.; Fiori, L.M.; Cruceanu, C.; Lin, R.; Labonte, B.; Cates, H.M.; Heller, E.A.; Vialou, V.; Ku, S.M.; Gerald, C.; et al. MicroRNAs 146a/b-5 and 425-3p and 24-3p are markers of antidepressant response and regulate MAPK/Wnt-system genes. *Nat. Commun.* **2017**, *8*, 15497. [CrossRef] [PubMed]
16. Hazra, D.; Kim, M.R.; Byun, Y.C. Generative Adversarial Networks for Creating Synthetic Nucleic Acid Sequences of Cat Genome. *Int. J. Mol. Sci.* **2022**, *23*, 3701. [CrossRef] [PubMed]
17. Pliatsika, V.; Loher, P.; Telonis, A.G.; Rigoutsos, I.J.B. MINTbase: A framework for the interactive exploration of mitochondrial and nuclear tRNA fragments. *Bioinformatics* **2016**, *32*, 2481–2489. [CrossRef] [PubMed]
18. Wei, R.; Wang, J.; Su, M.; Jia, E.; Chen, S.; Chen, T.; Ni, Y. Missing value imputation approach for mass spectrometry-based metabolomics data. *Sci. Rep.* **2018**, *8*, 663. [CrossRef] [PubMed]
19. Vlachos, I.S.; Zagganas, K.; Paraskevopoulou, M.D.; Georgakilas, G.; Karagkouni, D.; Vergoulis, T.; Dalamagas, T.; Hatzigeorgiou, A.G. DIANA-miRPath v3.0: Deciphering microRNA function with experimental support. *Nucleic Acids Res.* **2015**, *43*, W460–W466. [CrossRef] [PubMed]
20. Upadhyaya, H.P.; Arnold, L.M.; Alaka, K.; Qiao, M.; Williams, D.; Mehta, R. Efficacy and safety of duloxetine versus placebo in adolescents with juvenile fibromyalgia: Results from a randomized controlled trial. *Pediatr. Rheumatol. Online J.* **2019**, *17*, 27. [CrossRef]
21. Muscatello, M.R.A.; Zoccali, R.A.; Pandolfo, G.; Mangano, P.; Lorusso, S.; Cedro, C.; Battaglia, F.; Spina, E.; Bruno, A. Duloxetine in Psychiatric Disorders: Expansions Beyond Major Depression and Generalized Anxiety Disorder. *Front. Psychiatry* **2019**, *10*, 772. [CrossRef]
22. Gao, W.; Chen, R.; Xie, N.; Tang, D.; Zhou, B.; Wang, D. Duloxetine-Induced Neural Cell Death and Promoted Neurite Outgrowth in N2a Cells. *Neurotox. Res.* **2020**, *38*, 859–870. [CrossRef] [PubMed]
23. Dwivedi, Y. MicroRNAs in depression and suicide: Recent insights and future perspectives. *J. Affect. Disord.* **2018**, *240*, 146–154. [CrossRef]
24. Pan, B.; Liu, Y. Effects of duloxetine on microRNA expression profile in frontal lobe and hippocampus in a mouse model of depression. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 15454–15461. [PubMed]
25. Roy, B.; Dunbar, M.; Shelton, R.C.; Dwivedi, Y. Identification of MicroRNA-124-3p as a Putative Epigenetic Signature of Major Depressive Disorder. *Neuropsychopharmacology* **2017**, *42*, 864–875. [CrossRef]
26. Kim, H.K.; Tyryshkin, K.; Elmi, N.; Dharsee, M.; Evans, K.R.; Good, J.; Javadi, M.; McCormack, S.; Vaccarino, A.L.; Zhang, X.; et al. Plasma microRNA expression levels and their targeted pathways in patients with major depressive disorder who are responsive to duloxetine treatment. *J. Psychiatr. Res.* **2019**, *110*, 38–44. [CrossRef]
27. Yang, Y.; Yang, J.; Ma, T.; Yang, X.; Yuan, Y.; Guo, Y. The role and mechanism of TGF-beta1 in the antidepressant-like effects of tetrahydrocurcumin. *Eur. J. Pharmacol.* **2023**, *959*, 176075. [CrossRef]
28. Ogata, H.; Higasa, K.; Kageyama, Y.; Tahara, H.; Shimamoto, A.; Takekita, Y.; Koshikawa, Y.; Nonen, S.; Kato, T.; Kinoshita, T.; et al. Relationship between circulating mitochondrial DNA and microRNA in patients with major depression. *J. Affect. Disord.* **2023**, *339*, 538–546. [CrossRef]
29. Koskinen, M.K.; van Mourik, Y.; Smit, A.B.; Riga, D.; Spijker, S. From stress to depression: Development of extracellular matrix-dependent cognitive impairment following social stress. *Sci. Rep.* **2020**, *10*, 17308. [CrossRef]
30. Spijker, S.; Koskinen, M.K.; Riga, D. Incubation of depression: ECM assembly and parvalbumin interneurons after stress. *Neurosci. Biobehav. Rev.* **2020**, *118*, 65–79. [CrossRef]
31. Sutherland, T.E.; Dyer, D.P.; Allen, J.E. The extracellular matrix and the immune system: A mutually dependent relationship. *Science* **2023**, *379*, eabp8964. [CrossRef] [PubMed]

32. Zhao, J.; Liu, H.; Chen, Q.; Xia, M.; Wan, L.; Yu, W.; Liu, C.; Hao, X.; Tang, C.; Chen, G.; et al. Mechanistic study of celastrol-mediated inhibition of proinflammatory activation of macrophages in IgA nephropathy via down-regulating ECM1. *Int. J. Biol. Sci.* **2024**, *20*, 5731–5746. [CrossRef] [PubMed]
33. Chen, X.; Huang, C.; Sun, H.; Hong, H.; Jin, J.; Bei, C.; Lu, Z.; Zhang, X. Puerarin suppresses inflammation and ECM degradation through Nrf2/HO-1 axis in chondrocytes and alleviates pain symptom in osteoarthritic mice. *Food Funct.* **2021**, *12*, 2075–2089. [CrossRef]
34. Parisien, M.; Samoshkin, A.; Tansley, S.N.; Piltonen, M.H.; Martin, L.J.; El-Hachem, N.; Dagostino, C.; Allegri, M.; Mogil, J.S.; Khoutorsky, A.; et al. Genetic pathway analysis reveals a major role for extracellular matrix organization in inflammatory and neuropathic pain. *Pain* **2019**, *160*, 932–944. [CrossRef]
35. Tajerian, M.; Hung, V.; Nguyen, H.; Lee, G.; Joubert, L.M.; Malkovskiy, A.V.; Zou, B.; Xie, S.; Huang, T.T.; Clark, J.D. The hippocampal extracellular matrix regulates pain and memory after injury. *Mol. Psychiatry* **2018**, *23*, 2302–2313. [CrossRef] [PubMed]
36. Govil, N.; Arora, P.; Parag, K.; Tripathi, M.; Garg, P.K.; Goyal, T. Postoperative acute pain management with duloxetine as compared to placebo: A systematic review with meta-analysis of randomized clinical trials. *Pain Pract.* **2023**, *23*, 818–837. [CrossRef] [PubMed]
37. Nakamura, M.; Yoshimi, A.; Tokura, T.; Kimura, H.; Kishi, S.; Miyauchi, T.; Iwamoto, K.; Ito, M.; Sato-Boku, A.; Mouri, A.; et al. Duloxetine improves chronic orofacial pain and comorbid depressive symptoms in association with reduction of serotonin transporter protein through upregulation of ubiquitinated serotonin transporter protein. *Pain* **2024**, *165*, 1177–1186. [CrossRef]
38. Mockel, T.; Basta, F.; Weinmann-Menke, J.; Schwarting, A. B cell activating factor (BAFF): Structure, functions, autoimmunity and clinical implications in Systemic Lupus Erythematosus (SLE). *Autoimmun. Rev.* **2021**, *20*, 102736. [CrossRef]
39. Frost, E.; Hofmann, J.N.; Huang, W.Y.; Frazer-Abel, A.A.; Deane, K.D.; Berndt, S.I. Serum levels of B-cell activating factor are associated with a reduced risk of chronic lymphocytic leukemia. *Blood Cancer J.* **2024**, *14*, 132. [CrossRef] [PubMed]
40. Bahr, H.I.; Abdelghany, A.A.; Galhom, R.A.; Barakat, B.M.; Arafa, E.A.; Fawzy, M.S. Duloxetine protects against experimental diabetic retinopathy in mice through retinal GFAP downregulation and modulation of neurotrophic factors. *Exp. Eye Res.* **2019**, *186*, 107742. [CrossRef]
41. Li, E.; Yin, H.; Su, M.; Li, Q.; Zhao, Y.; Zhang, L.; Guo, J.; Lai, X.; Xue, X.; Tang, C. Inhibition of ferroptosis alleviates chronic unpredictable mild stress-induced depression in mice via tsRNA-3029b. *Brain Res. Bull.* **2023**, *204*, 110773. [CrossRef]
42. Tian, H.; Gao, S.; Xu, M.; Yang, M.; Shen, M.; Liu, J.; Li, G.; Zhuang, D.; Hu, Z.; Wang, C. tiRNA-Gly-GCC-001 in major depressive disorder: Promising diagnostic and therapeutic biomarker. *Br. J. Pharmacol.* **2024**, *181*, 1952–1972. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.