Systematic Analysis of tRNA-Derived Small RNAs Reveals Effects of Xuefu-Zhuyu Decoction on Hippocampus of Rats after Traumatic Brain Injury

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Systematic Analysis of tRNA-Derived Small RNAs reveals effects of Xuefu-Zhuyu decoction on hippocampus of rats after traumatic brain injury

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ABSTRACT

Background: Traumatic brain injury (TBI) is one of the most common neurosurgical diseases which refers to brain function impairment or brain pathological changes induced by external causes. A traditional Chinese medicine, Xuefu Zhuyu Decoction (XFZYD), has been indicated to harbor therapeutic property against TBI. Transfer RNA (tRNA)-derived small RNAs i.e., tsRNAs (a group of small RNAs derived from tRNAs) are multifunctional regulatory non-coding RNAs generated under pressure and implicated in the progression of TBI.

Methods: TBI model was successfully constructed by using of rats. Further using sequencing and omics to identify novel tsRNAs as drug targets for XFZYD therapy against TBI in rat hippocampus. qPCR assay was used to further verify the experimental results. GO analyzed the signaling pathways of downstream target genes of tsRNA in XFZYD regulated TBI model. qPCR was used to detect the influence of over-expressed tsRNA mimic/inhibitor on their target genes in PC12 cell.

Results: Our RNA-Seq data illustrates that 11 tsRNAs were mediated by the XFZYD. The experimental data revealed AS-tDR-002004 and AS-tDR-002583 as potential targets for XFZYD therapy and influenced TBI via the cadherin signaling pathway, cocaine addiction, circadian entrainment and nicotine pharmacodynamics pathway. We also confirm that Pi4kb, Mlh3, Pcdh9, and Ppp1cb were targets genes of 2 XFZYD regulated tsRNAs in hippocampus of rat model and PC12 cells. Furthermore, biological function analysis revealing potential therapeutic effects of tsRNAs, and results found Mapk1, Gnai1 was the related genes of for XFZYD therapy against TBI.

Conclusion: Our work successfully illuminates the efficiency of XFZYD for the treatment of TBI. The experimental data revealed AS-tDR-002004 and AS-tDR-002583 as potential targets for XFZYD therapy and influenced TBI via the cadherin signaling pathway, cocaine addiction, circadian entrainment and nicotine pharmacodynamics pathway in TBI rat model.

INTRODUCTION

Traumatic brain injury (TBI) is a global public health problem characterized by brain function impairment or brain pathological changes induced by external causes[1]. Being one of the most common neurological diseases, TBI can lead to temporary or even permanent nerve dysfunction in the brain[2]. In addition to the acute onset and rapid progress, TBI is also featured with high mortality and disability rate. Globally, TBI results in approximately 10 million hospitalizations and/or deaths every year with an estimated number of 57 million people still suffering the consequences of the condition[3]. Unfortunately, it remains challenging to clinically improve the survival rate of patients with TBI and to promote the recovery of nerve function. However, the effects of currently available treatment modalities on the recovery of nerve function are limited[4, 5]. TBI is a diverse group of sterile injuries caused by primary and secondary mechanisms that contribute to cell death, inflammation, and neurologic dysfunction in patients of all demographics[5, 6]. The primary injury of TBI has attributed to mechanical stress or shear force yet therapeutic drugs are not available at present. The secondary injury of TBI involves multiple processes and the current therapeutic strategies include activation of inflammatory and immune response, calcium overload, glutamate toxicity, and mitochondrial dysfunction[5, 7, 8]. The drugs against secondary injury of TBI include free-radical scavengers, antagonists of N-methyl-D-aspartate, and calcium channel blockers[5, 9-11]. Nevertheless, the outcomes are not effective. Thereby, it is urgent to develop novel therapeutic strategies and drugs for the management of TBI. The traditional Chinese medicine, Xuefu Zhuyu Decoction (XFZYD), can invigorate the circulation of blood without any consumption of blood as well as dissipate blood stasis and promote angiogenesis[5, 12]. Oral administration leads to peripheral vasodilation and enhanced aerobic catabolism, alleviating tissue edema, exudation, and inflammatory reaction by which clinical symptoms can be improved[5, 13, 14]. Recently reported research work has demonstrated the treatment effects of XFZYD on TBI yet the underlying mechanism remains unknown. Transfer RNA (tRNA)-derived small RNAs (tsRNAs), also known as tRNA-derived fragments (tRFs),

are a group of small RNAs derived from tRNAs that are multifunctional regulatory inhomogeneous small non-coding RNAs (ncRNAs) with a length of 18-40 nucleolus[15, 16]. It was primarily considered as by-products of randomly-cleaved tRNAs. According to the length and cleavage site, tsRNAs usually can be grouped into tRFs and tRNA halves (tiRNAs), most of which is generated under pressure[15, 16]. TsRNAs harbor potential regulatory functions implicated in diverse biological and pathological processes. Recent investigations have emerged regarding new functions of tsRNAs based on tsRNA sequences, RNA modification, and structure[5, 17, 18]. The involvement of tsRNAs in tumorigenesis has been indicated in different contexts, including chronic lymphocyte leukemia, lung cancer, breast cancer, and ovarian cancer[5, 19, 20]. Moreover, tsRNAs have been recognized to play important roles in spermatogenesis, differentiation, and metabolic process[21]. Additionally, omics has provided technical means for the investigation of tsRNAs in nerves and brains[5, 22]. Notably, some tsRNAs have been revealed to participate in the mediation of neurological disorders and pathological processes of traumatic spinal cord injury. Li et al. has proposed another traditional Chinese medicine, Buyang-Huanwu-Decoction, as a new therapeutic target for cerebral hemorrhage based on a system analysis of tsRNAs[23]. We endeavored to identify target tsRNAs of the traditional Chinese medicine, XFZYD, for treatment of TBI and to unravel the underlying mechanism, offering a promising candidate for management of TBI.

METHODS

Preparation of XFZYD

The matching prescription of XFZYD was as follows：Peach seeds were 12g; safflower, angelica sinensis, rehmannia root 9g, respectively; ligusticum chuanxiong and platycodon were 4.5g, respectively, red peony root, fructus aurantii and licorice root were 6g, respectively, and bupleurum root was 3g. The plant seeds and tissue were obtained commercially from the pharmacy of Xiangya Hospital, Central South University. Plant material used in this study were on sale in the pharmacy of Xiangya

Hospital, which is according with the 10th Pharmacopoeia Commission of the People's Republic of China.

Traumatic brain injury (TBI)

All animal protocols were approved by the Committee on the Use and Care of Animals of CSU and conformed to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication No. 85-23, revised 1996). The protocol was approved by the Medical Ethics Committee of Xiangya Hospital of Central South University. Adult male Sprague-Dawley rats (180-220 g) were obtained from Hunan SJA Laboratory Animal Co., Ltd (SCXK (XIANG) 2019-0004), and housed in SPF condition of the Laboratory Animal Centre of Central South University (CSU, SYXK (XIANG) 2015-0017). Rats were randomly divided into three groups: sham, CCI and XFZYD group and 4 rats per groups. CCI was performed as described previously under 3% pentobarbital sodium (60 mg/kg) anesthesia[24]. The parameters were as follows: impact depth, 5.0 mm; striking speed, 6.0 m/sec; dwell time, 50 msec. The sham-operated rats were subjected to the same anesthesia and craniotomy except for cortical impact. In the XFZYD group, rats were intragastrically administrated with 1.52 g/kg (equivalent to 9 g/kg of raw herbs) XFZYD. The rats in the sham and CCI groups were treated with equal volumes of distilled water mNSS was used to assess movement, sensation, and reflexes of TBI rats.

tsRNA Sequencing (tsRNA-Seq)

Rats were deeply anesthetized and sacrificed by intraperitoneal injection of pentobarbital and perfused with ice-cold saline. Then, hippocampus tissues surrounding the hemorrhagic region were harvested for following detections. Total RNAs were extracted from sham, TBI and XFZYD groups (n=4 each group) according to the manufacturer’s instruction (Qiagen, USA). Subsequently, using rtStar tRF& tiRNA Pretreatment Kit protocols (Arraystar, USA), some RNA modifications which might interfere with small RNA-sequencing library construction were removed

(Fig. 1). cDNA was then synthesized and amplified using Illumina's proprietary reverse transcription primers and amplification primers. Afterwards, 135-170 nt PCR amplified fragments (corresponding to 15-50 nt small RNA) were extracted and purified from the PAGE gel using an automated gel cutter. The libraries are qualified and absolutely quantified using Agilent BioAnalyzer 2100. Finally, the sequencing run was performed on Illumina NextSeq 500 system using NextSeq 500/550 V2 kit (Illumina, USA). Sequencing was carried out by running 50 cycles. RNA-seq data has been submitted in NCBI: SUB8928870.

Data analysis

The raw sequencing data that passed the Illumina chastity filter were used for the following analysis. After Illumina quality control, the sequencing reads were 5', 3'-adaptor trimmed, filtered for over 15 nt by Cutadapt software. And then using NovoAlign software (v2.07.11), trimmed reads were aligned to mature-tRNA and pre-tRNA sequences from GtRNAdb (http://gtrnadb.ucsc.edu/). The exactly matched reads were thought as tsRNAs. Moreover, tsRNA expression levels could be measured and normalized as tag counts per million of total aligned tRNA reads (TPM). tsRNA expression profiling and differential expression analysis were calculated by the average TPM. Fold changes (FC; TBI versus sham or XFZYD versus TBI) were used for comparing two groups of profile differences. In a ddition, FC>1.3 and P-value <0.05 were considered significantly different expression and these tsRNAs were chosen for further analysis. In the standard of FC>1.3 and P<0.05, we could identify TBI-induced tsRNAs (TBI vs sham) and XFZYD-induced tsRNAs (XFZYD vs TBI).

Target Prediction of treatment-related tsRNAs

Used two common algorithms to predict tsRNA targets, namely, TargetScan (http://www.targetscan.org), miRanda (http://www.microrna.org). Additionally, to reduce false-positive results, only genes predicted by all four software were considered as targets of tsRNAs. The network illustration was visualized with Cytoscape software (version 3.5.1, the Cytoscape Consortium, San Diego, CA, USA).

Gene Ontology (GO) and Pathway Enrichment Analysis. The functional enrichment tool DAVID (DAVID, https://david.ncifcrf.gov/), ver. 6.8) was used to calculate both the KEGG pathway and GO biological processes (BP) enrichment.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol extraction reagents (Invitrogen, Grand Island, NY, USA), according to the methods of manufacturer's instructions. RNA purity and concentration were photometrically tested. RNA was reverse-transcribed into cDNA using Reverse Aid First Strand cDNA Synthesis Kit (Takara, Japan). qPCR was performed using SYBR Green qPCR Supermix (Takara, Japan) with ABI 7500 RT-PCR (Bio-Rad, CA, USA). The gene expression levels were calculated relative to β-actin using the $2^{-\Delta\Delta Cq}$ method. The cycling conditions were as follows: incubation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s, 60 °C for 60 s and 95 °C for 15 s. The primer sequence is as follows:

β-actin F: 5' ACATCCGTAAAGACCTCTATGCC 3', R: 5' TACTCCTGCTTGC TGATCCAC 3';

Pdyn F: 5' CACGGAACTGACCAAGCTCT 3', R: 5' GTCAGTGCCCAGT AGCTCAG 3';

Mapk1 F: 5' TGAAGACACAGCACCTCAGCAATG 3', R: 5' GGTGTTCAGC AGGAGGTTGGAAG 3';

Pcdh9 F: 5'GTGCTTGGTTTTGGGTCACT 3', R: 5' CGGTCATTGAACTGGTT CCT 3';

Gnai1 F: 5' GTGCTTGGAGCCCGCACTCGG 3', R: 5' AGATTCACCAGCAC CGAGCAGCA 3';

Pi4bk F: 5'GCCCACCAGGGAATAA3', R: 5' TCCACTACTGTATCTCCCAT3';

Mhl3 F: 5' GACGTATGTTCCCGATTTTGTCA 3', R: 5' GCTTCAGAGCTGAT ATAGCCACT 3';

Ppp1cb F: 5’ TGGACAGCCTCATCAC 3’, R: 5’ TTCAGCTCCCCGTCCGCCA T 3’.

Cell culture

HyClone™ Dulbecco’s modified Eagle’s medium (DMEM) (GE Healthcare Life Science) with 10 % fetal bovine serum (FBS) (Corning) was used for PC12 cells culture. The cell lines of PC12 were bought from American Type Culture Collection (ATCC, USA). Cells were maintained at 37 °C in a humidified incubator with 5% CO2 with cell passage performed, when cell density was ~ 90%.

Statistical analysis

All results are expressed as the mean ± SE. Statistical analysis of the data was performed using GraphPad Prism 7 (Sorrento Valley, CA, USA). Comparisons between samples were performed by one-way ANOVA with Tukey's multiple comparison tests. Differences were considered significant at $p < 0.05$.

RESULTS

Effects of XFZYD on neurological recovery after TBI

The laboratory rats were divided into 3 groups, the sham group, the TBI group, and the XFZYD group (n=4 each group). The degree of nerve injury was assessed by the modified neurological severity score (mNSS) and weight changes. On 0 day, mNSS scores were performed to show the base line levels of normal rats and there was no statistically significance between the three groups ($P>0.05$) (Fig. 2A). Compared with the sham group, the mNSS scores of the 1st day was significantly higher in TBI group while weight change was remarkably decreased in the TBI group ($P < 0.001$) (Fig. 2B), indicating the successful establishment of a rat model of TBI. To determine whether long-term XFZYD therapy was beneficial to TBI in rats, XFZYD therapy was applied to rats with TBI for 1–21 days. On the 21st day, the scores of TBI rats were still higher than sham in mNSS ($P<0.01$) (Fig. 2A). However, after 21 days treament of XFZYD, the scores of XFZYD rats significantly declined in mNSS ($P<0.05$). Additionally, TBI

worsened the weight growth than sham (P<0.001). And XFZYD treatment could improve rise over TBI (P<0.05) (Fig. 2B). Taken together, these findings suggested that long-term XFZYD therapy significantly alleviated rat behaviors during recovery when compared with the TBI group. Therefore, the following experiments were performed using 21-day therapy of XFZYD.

XFZYD therapy changed the expression profile of tsRNAs

For identification of targets of XFZYD therapy against TBI, tsRNA-Seq method was introduced to detect the expression profile changes of tsRNAs in rat hippocampus of the sham, TBI, and XFZYD groups. The original data of tsRNA-Seq had been submitted to Gene Expression Omnibus. There were 365 precisely matched tsRNAs identified from tsRNA-Seq, 322 for the sham group, 51 for the TBI group, and 197 for the XFZYD group (Fig. 3A and Additional file 1). Generally, tsRNA expression was significantly increased in the TBI group to various degrees than that in the sham group. Although rats following XFZYD therapy could not match up to rats in the sham group, however, changes induced by TBI were slightly reversed (Fig. 3B). When comparing the TBI group with the sham group, expression levels of 322 tsRNAs was altered. In comparison to the TBI group, expression levels of 51 tsRNAs changed in the XFZYD group. Hence, 41 tsRNAs were obtained in the intersection that might respond to XFZYD therapy against TBI (Fig. 3C). Additionally, we identified significantly dysregulated tsRNAs in TBI rats: 5 were up-regulated, while 7 were down-regulated (TBI vs sham; FC > 1.3 and $p < 0.05$) (Fig. 3D and Additional file 2). And after XFZYD treatment, 11 tsRNAs were obviously changed: 10 were upregulated, while 1 was down- regulated (XFZYD vs TBI; FC > 1.3 and $p < 0.05$) (Fig. 3D).

Identification and confirmation of related tsRNAs by XFZYD therapy

Afterward, we aimed to identify the significantly up-regulated/down-regulated tsRNAs in the TBI group than those in the sham group as well as significantly down-regulated/up-regulated tsRNAs in the XFZYD group than those in the TBI group. Accordingly, 31 tsRNAs were found as shown by heat maps in Fig. 4AB. After

241 screening, one up-regulated tsRNA (AS-tDR-013642) and 30 down-regulated tsRNAs
242 (TBI vs. sham; Fold Change > 1.5, $p < 0.05$) were selected (Additional file 2).
243 Moreover, 11 tsRNAs were finally obtained with tag count ≠ 0 as the threshold since
244 sequences from tsRNA-Seq were required to be preciously matched with those of
245 derived tsRNAs, including 10 up-regulated tsRNAs (AS-tDR-001612,
246 AS-tDR-002004, AS-tDR-002356, AS-tDR-002372, AS-tDR-002583,
247 AS-tDR-004117, AS-tDR-004118, AS-tDR-006440, AS-tDR-013227 and
248 AS-tDR-013228) and one down-regulated tsRNA (AS-tDR-013642) (XFZYD vs. TBI;
249 Fold Change > 1.5, $p < 0.05$).

250 Furthermore, a quantitative polymerase chain reaction (qPCR) was conducted for
251 verification of the selected 11 tsRNAs (Fig. 4C). AS-tDR-002004 and AS-tDR-002583
252 were significantly down-regulated in the TBI group than that in the sham group ($p <$
253 0.05). After XFZYD therapy, AS-tDR-002004 and AS-tDR-002583 were significantly
254 upregulated ($p < 0.01$). Conclusively, these results revealed that AS-tDR-002004 and
255 AS-tDR-002583 as potential targets of XFZYD therapy against TBI. Identification and
256 confirmation of related target genes of tsRNAs by XFZYD therapy Subsequently,
257 target genes of AS-tDR-002004 and AS-tDR-002583 were analyzed by Targetscan, the
258 results of which revealed 310 and 90 transcripts of AS-tDR-002004 and
259 AS-tDR-002583 respectively (Fig. 5A and Additional file 3). Two common target
260 genes Pi4kb and Mlh3 were identified (Fig. 5B). The binding sites are depicted in Fig.
261 5C. Thereafter, the qPCR assay was performed to verify that during XFZYD therapy,
262 Pi4kb and Mlh3 were the related target genes of tsRNAs. Our results showed that both
263 Pi4kb and Mlh3 were upregulated in the TBI group and downregulated after XFZYD
264 therapy when compared with the sham group ($p < 0.05$) (Fig. 5D). Whilst the resulting
265 tsRNA expression was changed oppositely ($p<0.05$). Additionally, tsRNAs were
266 overexpressed or inhibited in the PC12 cell line to explore the expression alternation of
267 their target genes for further verification (Fig. 5E and F). After transfection of
268 AS-tDR-231002004 mimic or AS-tDR-002583 mimic, expression of Pi4kb and Mlh3
269 was diminished ($p < 0.05$) while the delivery of AS-tDR-002004 inhibitor or

AS-tDR-002583 inhibitor was significantly elevated expression of Pi4kb and Mlh3 ($p < 0.05$) (Fig. 5E and F). These in vitro results were consistent with qPCR results in a rat model. The above-mentioned findings demonstrated that AS-tDR-002004 and AS-tDR-002583 might be involved in XFZYD therapy against TBI by regulating their common target genes i.e., Pi4kb and Mlh3.

Analysis of target genes of XFZYD therapy-related tsRNAs

Despite that detailed regulatory mechanism of tsRNAs remains less-studied, a recent surge of evidence has proved the similar function between tsRNAs and microRNAs considering the transcription inhibitory action. Yet seed sequences can potentially inhibit the overall translation activity of target messenger RNAs by complementary base pairing. Targetsan and miRanda databases were used to predict 11 XFZYD-related target genes of selected 11 tsRNAs, including AS-tDR-001612, AS-tDR-002004, AS-tDR-002356, AS-tDR-002372, AS-tDR-002583, AS-tDR-004117, AS-tDR-004118, AS-tDR-006440, AS-tDR-013227, AS-tDR-013228, and AS-tDR-013642. Thus, all target genes of 11 tsRNAs were identified and analyzed through an intersection as shown in Fig. 6A and Additional file 4.

According to the following table(Fig. 6B), 30 target genes were identified through the intersection of target genes of 2 random-selected tsRNAs from 11 tsRNAs in Fig. 6A. Gene Ontology (GO) pathway analysis was conducted on the 30 genes as shown in Fig. 6B. Target genes participated in the Alzheimer's disease-presenilin pathway, Nicotine pharmacodynamics pathway, Toll receptor signaling pathway, Wnt signaling pathway, and cadherin signaling pathway, among which Alzheimer's disease and Huntingdon disease belong to neurological diseases, further supporting the validation of our results. Two genes (Pcdh9 and Ppp1cb) were revealed from the intersection between the selected signaling pathways and target genes of AS-tDR-002004 or AS-tDR-002583. In other words, Pcdh9, the target gene of AS-tDR-002004, and Ppp1cb, the target gene of AS-tDR-002583, both were involved in the cadherin signaling pathway and Nicotine pharmacodynamics pathway, respectively. Following

after we investigate the effects of AS-tDR-002004 or AS-tDR-002583 on the expression of Pcdh9 and Ppp1cb. It was found that AS-tDR-002004 mimic inhibited the Pcdh9 expression and AS-tDR-002583 mimic suppressed the Ppp1cb expression (Fig. 6C). Hence, these results indicated that AS-tDR-002004 or AS-tDR-002583, as targets of XFZYD therapy for TBI, alleviating TBI via the cadherin signaling pathway and Nicotine pharmacodynamics pathway.

Biological Function Analysis Revealing Potential Therapeutic Effects of tsRNAs

The tsRNAs could regulate mRNA translational activities, and hence, to understand their biological functions, we conducted a bioinformatics analysis of the functions of the target genes. KEGG pathway was executed to explore the functions of target genes. According to KEGG enrichment analysis, Pertussis, Cocaine addiction, Gastric acid secretion, collecting duct acid secretion, Intestinal immune network for IgA production, Adrenergic signaling in cardiomyocytes, Glutamatergic synapse, Circadian entrainment, Apelin signaling pathway, Renin secretion etc. Moreover, cleavage of growing transcript in the termination region (conducted; 6 genes enriched) was the main term conducted by Reactome Gene Sets. Ranked by P-values, top 10 enriched terms were shown in Fig. 7A and Additional file 5. Among those, cocaine addiction and circadian entrainment was the related pathway of brain function.

There were 13 genes enriched in cocaine addiction and circadian entrainment signaling pathway (Bdnf, Gnai1, Gnai3, Grin2d, Grm2, Maob, Cam2, Camk2g, Gng12, Mapk1, Pcb4, Prkm2 and Pdyn). We analyzed these 13 genes and the target genes of AS-tDR-002004 or AS-tDR-002583 in Fig. 6B with the signaling pathways involved in these genes (Fig. 7B). These genes were regulated by 8 tsRNAs. Where Mapk1 was the target gene of AS-tDR-002004, and Gnai1 was the target gene for AS-tDR-002583. After over-expressing AS-tDR-002004 mimics in PC12 cells, Pcdh9, Mapk1 were significantly down-regulated ($P<0.01$, $P<0.05$), and Pdyn had no change (all $P>0.05$) (Fig. 7C). When transfected with AS-tDR-002583 mimics, GNAI1 was significantly downregulated ($P<0.05$) (Fig.7C).

Discussion

Traumatic brain injury (TBI) is a growing public health problem worldwide and is a leading cause of death and disability[25]. Although major progress has been made in understanding the pathophysiology of this injury, this has not yet led to substantial improvements in outcome by a lack of treatments which have proven successful during phase III trials for modern medicine[26]. XFZYD has been used for years to treat TBI in China and has been demonstrated to be effective in clinical practice[27]. However, the underlying mechanism remains unknown. The previous studies merely partially deciphered the molecule mechanism of XFZYD for treating TBI. The present study was intended to explore the expression profile of tsRNAs in a rat model of TBI before and after XFZYD therapy. Briefly, our RNA-Seq data illustrates that 11 tsRNAs were mediated by the XFZYD. While our qPCR assay validated that 2 tsRNAs (AS-tDR-002004 and AS-tDR-002583) were the potential targets for XFZYD therapy against TBI. Thereafter, we aimed to analyze the corresponding target genes i.e., Pi4kb and Mlh3. Intriguingly, our results identified a reciprocal relation between expression levels tsRNAs and these two target genes in the rat model. For further verification, 11 targeted genes of tsRNAs were analyzed and 30 intersected target genes were manifested by our results. Additionally, GO analysis showed signaling pathways related to neurologic disorders and adhesion, further making our results convincible. Subsequently, Pcdh9 was found to be correspondent to AS-tDR-002004 while Ppp1cb was corresponding to AS-tDR-002583 through the intersection between 30 target genes and target genes of 2 tsRNAs (AS-tDR-002004 and AS-tDR-002583). Lastly, the 2 tsRNAs were overexpressed or inhibited in PC12 cells to determine the resulting expression alternations of Pi4kb, Mlh3, Pcdh9, and Ppp1cb. AS-tDR-002004 and AS-tDR-002583 were confirmed as targets for XFZYD therapy against TBI that could alleviate TBI through the cadherin signaling pathway and Nicotine pharmacodynamics pathway. Furthermore, biological function analysis revealing potential therapeutic effects of tsRNAs, and results found Mapk1, Gnai1 was the related genes of for XFZYD therapy against TBI. Most of the human genome is

composed of ncRNAs that are extensively involved in diverse biological and pathologic activities. tsRNAs are the most common type of small ncRNAs and have been reported to participate in the modulation of RNA processing and protein translation[5, 28]. Mounting evidence has shed light on the close association between tsRNAs and various biological processes as well as human diseases, including tumors, diseases of the cardiovascular system, epigenetics, and neurologic disorders[29]. Ramos et al. have reported that remarkable number of neurodevelopmental disorders have been linked to defects in tRNA modifications[30]. Karaiskos et al. have also detected that tRFs originating from 3'- and 5'-ends of tRNAs in rat brains at significant levels, and illustrated the utility of tRF analysis for annotating tRNA genes in sequenced genomes[31]. However, the mechanism of tsRNAs in TBI remains enigmatic. Thus, our results further clarify the mechanisms of tsRNAs in TBI.

TBI is a growing public health problem worldwide and is a leading cause of death and disability[32]. Neuroscientists and surgeons tend to search for potential novel drugs from traditional Chinese medicine libraries to treat TBI[33]. The traditional Chinese medicine “XFZYD” possesses the ability to improve blood circulation and disperse stasis while it has also been elucidated as a reliable and effective therapy for multiple diseases, including unstable angina pectoris, coronary artery diseases, thromboembolic stroke, ischemic stroke, and TBI[5, 12]. It is mainly composed of flavonoids, organic acid, terpenoids, steroid saponins, etc. It has been reported that XFZYD is potentially functional in the alleviation of TBI from anti-depression and synaptic regulation perspectives, which are concordant with our study[34]. However, research regarding the detailed biological mechanism of XFZYD underlying TBI is in the incipient stage. Our work further authenticated the alleviating effects of XFZYD therapy in rats with TBI and identified the corresponding drug targets, establishing a foundation to reveal the pharmacological mechanism and offering evidence for clinical application of integrative medical treatment in therapy using Traditional Chinese and Western medicines.

Conclusions

Our work successfully illuminates the efficiency of XFZYD for the treatment of TBI. This study initiatively revealed the changed expression patterns of tsRNAs in hippocampus of CCI rats after XFZYD treatment. tsRNAs might be novel potential therapeutic targets for XFZYD to regulate TBI-induced biological pathways. The present study provides the basis and direction for future investigations to explore the mechanisms by which XFZYD protects against long-term neurological deficiencies after TBI. The present work may provide valuable evidence for further clinical application of XFZYD for treating TBI. The interaction process between tsRNAs and mRNAs are needed to be clarified clearly by more studies.

AUTHOR CONTRIBUTIONS

F. D., and P.G. conceived and initially designed the study. P.G., F.D., T.T. analyzed data, wrote, proofread, and revised the manuscript. F.D., T.T., R.L. conducted the TBI model, qPCR assay. P.L., D.F., M.H. conducted the GO, KEGG analysis. D.F., Y.W. conducted data analysis. All authors participated in data interpretation, reviewed, and approved the manuscript.

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Abbreviations

tRNA, tRNA-Derived Small RNAs；XFZYD, Xuefu-Zhuyu decoction; TBI, traumatic brain injury; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology； CCI, controlled cortical impact;

Availability of data and materials

Specific study data are available from the authors on request.

Ethics approval and consent to participate

The animal experiments were approved by the Animal Care and Use Committee of Xiangya Hospital, Central South University.

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Not Applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests in any aspects.

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FIGURES and LEGENDS

Figure 1. Study design illustration. TBI, traumatic brain injury; XFZYD, xuefu-zhuyu decoction; FC, fold change; qPCR, quantitative real-time PCR.

Figure 2. Effects of XFZYD on neurological recovery after TBI. On 1 day, the mNSS (A) weight change (B) of TBI and XFZYD group were significantly different with sham group, which indicated the successful TBI models. On 21 day, the mNSS (A) and weight change (B) of sham group and XFZYD group showed significantly different values than TBI group, which suggested the therapeutic effects of XFZYD for functional recovery after TBI. Data were presented as mean ± SEM (n=15 each group), * $p<0.05$, ** $p<0.01$, *** $p<0.001$ indicate significant difference compared with shame group; # $p<0.05$ indicate significant difference compared with TBI group, mNSS, modified neurological severity score; 0d, the day of surgery but before anesthesia; 1d and 21d, the 1st and 21st day after TBI; weight change, the difference between weight values of 1& 21 day and 0 day.

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mimics.

Additional file 1. The total precisely matched tsRNAs identified from tsRNA-Seq.

(A) indicated shame group; (B) indicated TBI group; (C) indicated XFZYD group.

Additional file 2. The significantly dysregulated tsRNAs from tsRNA-Seq.

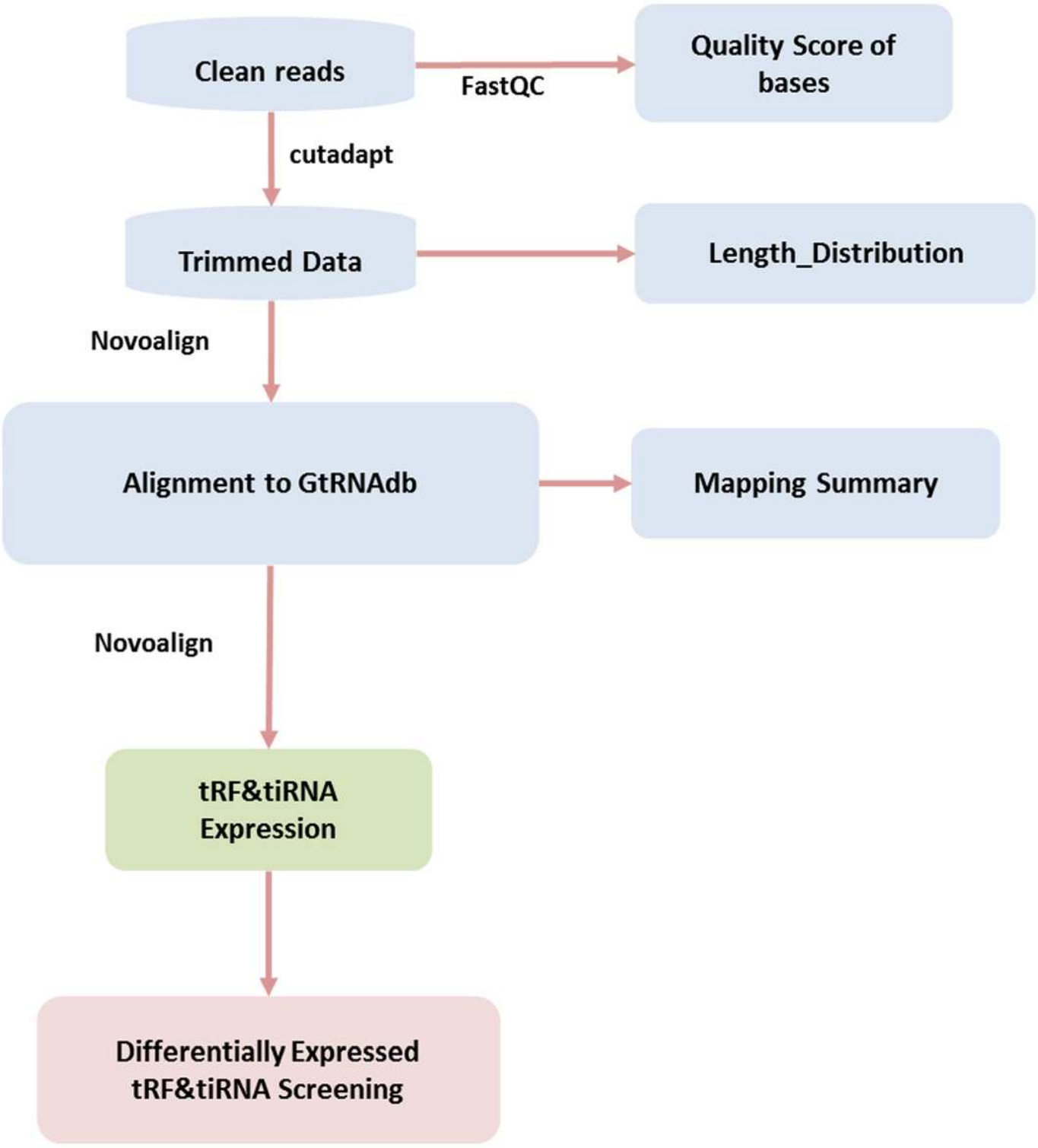
(A) indicated shame group; (B) indicated TBI group; (C) indicated XFZYD group.($FC > 1.3$ and $p < 0.05$).

Additional file 3. Target genes of AS-tDR-002004 and AS-tDR-002583.

Additional file 4. Identified and analyzed all target genes of 11 tsRNAs.

Additional file 5. Top 10 enriched terms which ranked by P-values.

Figures



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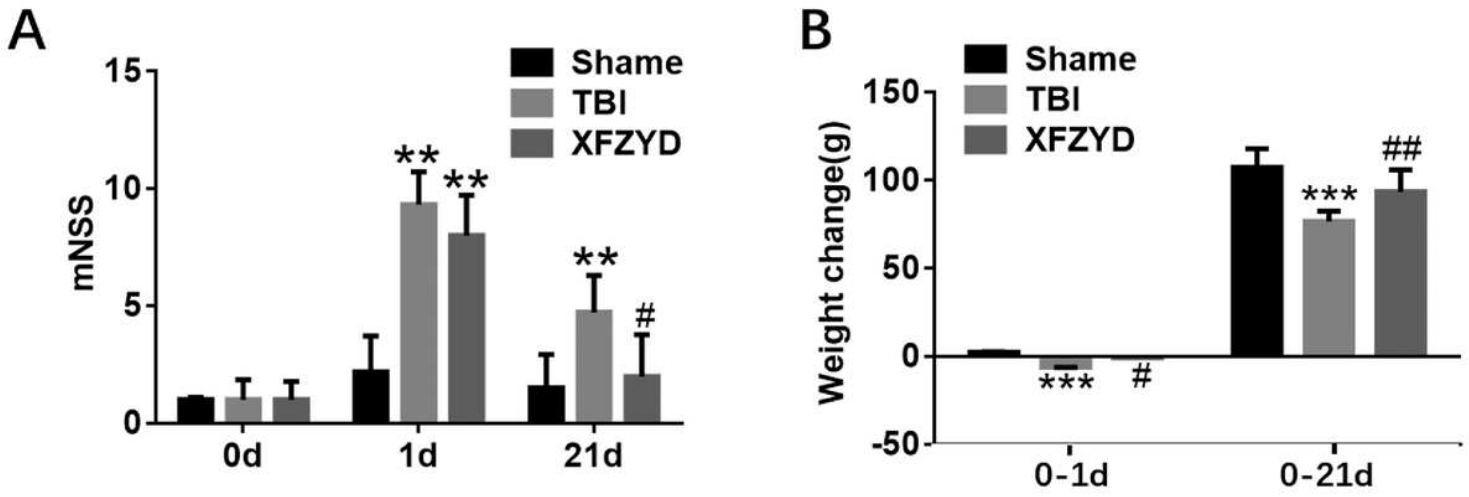

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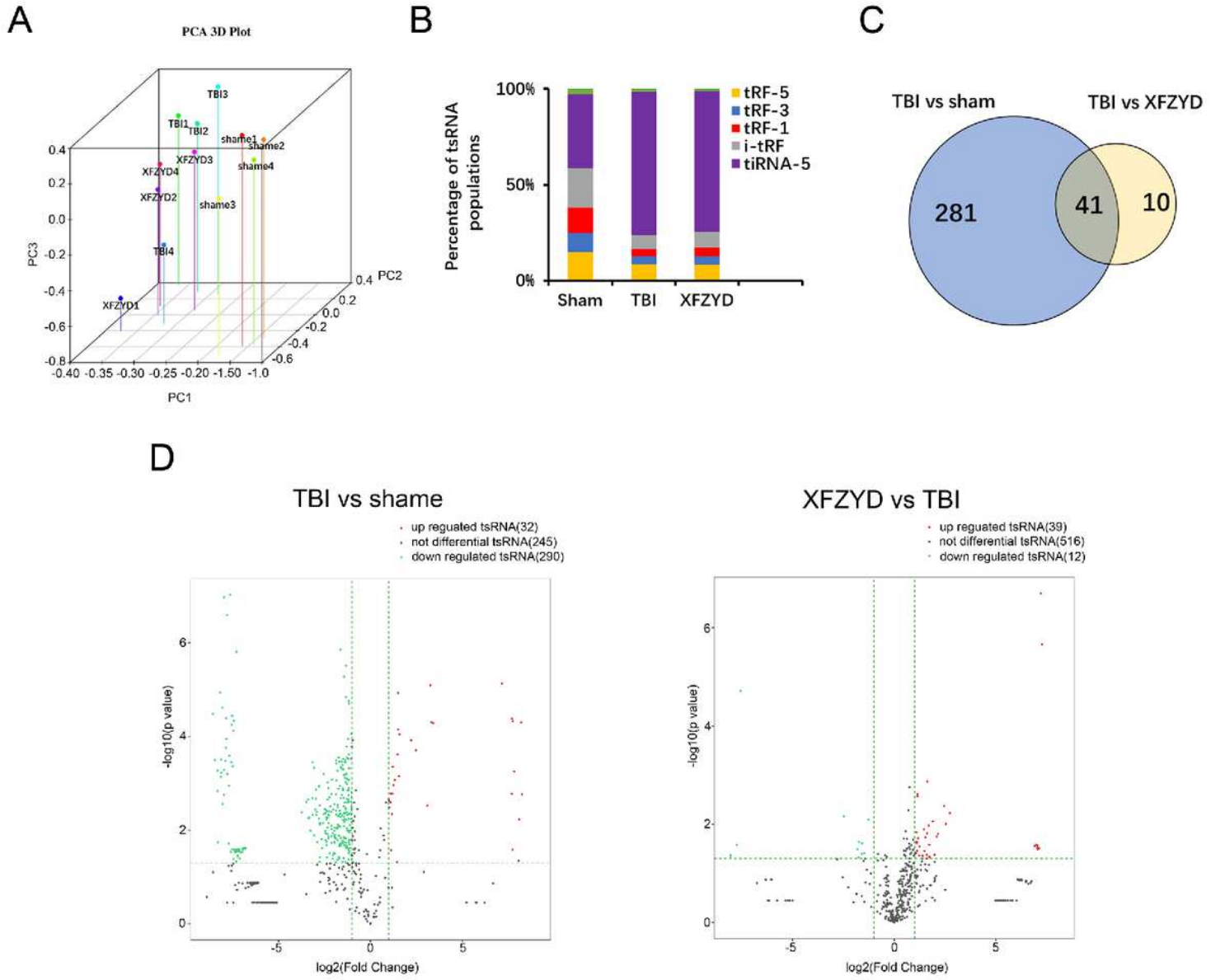

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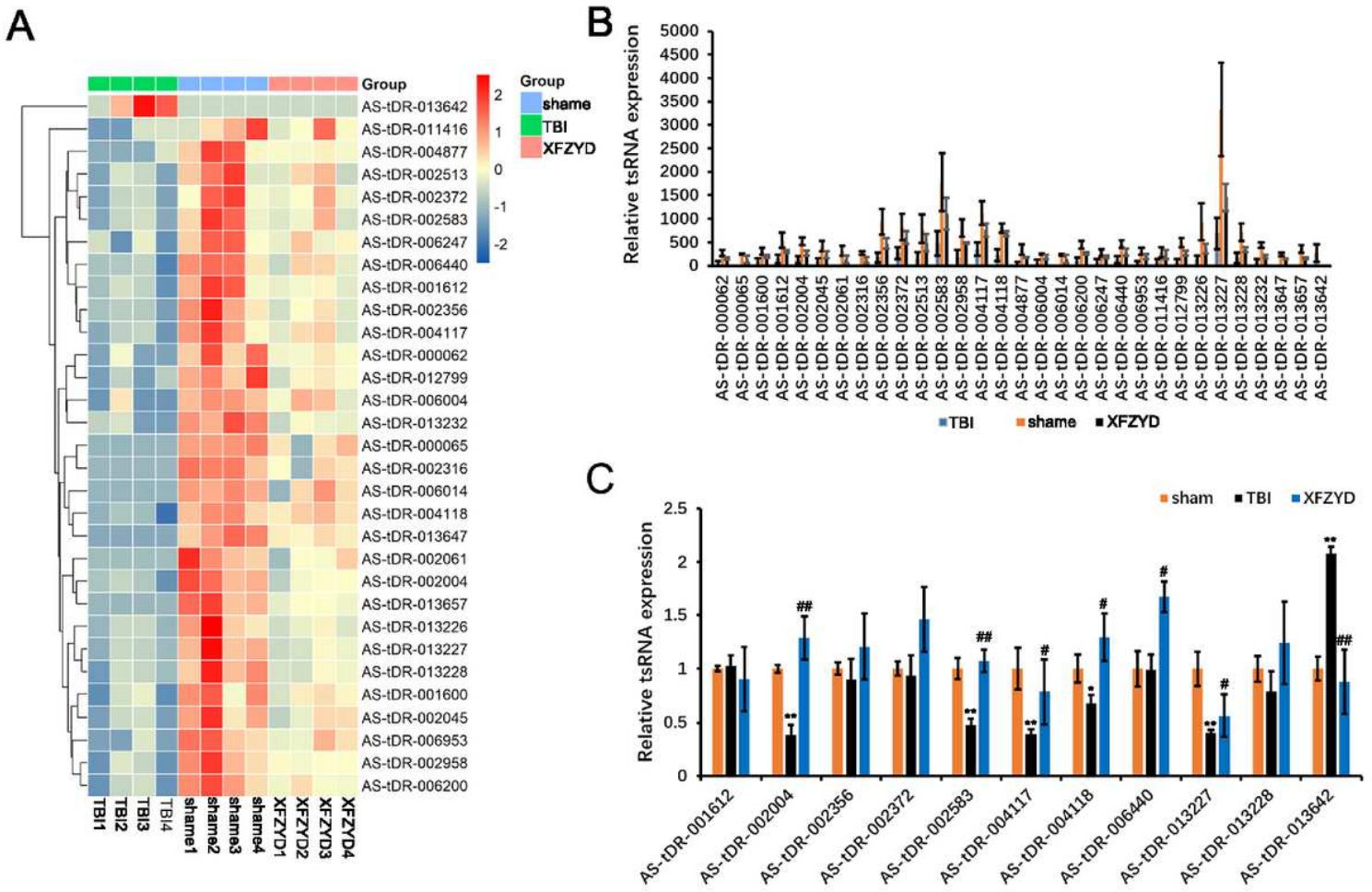

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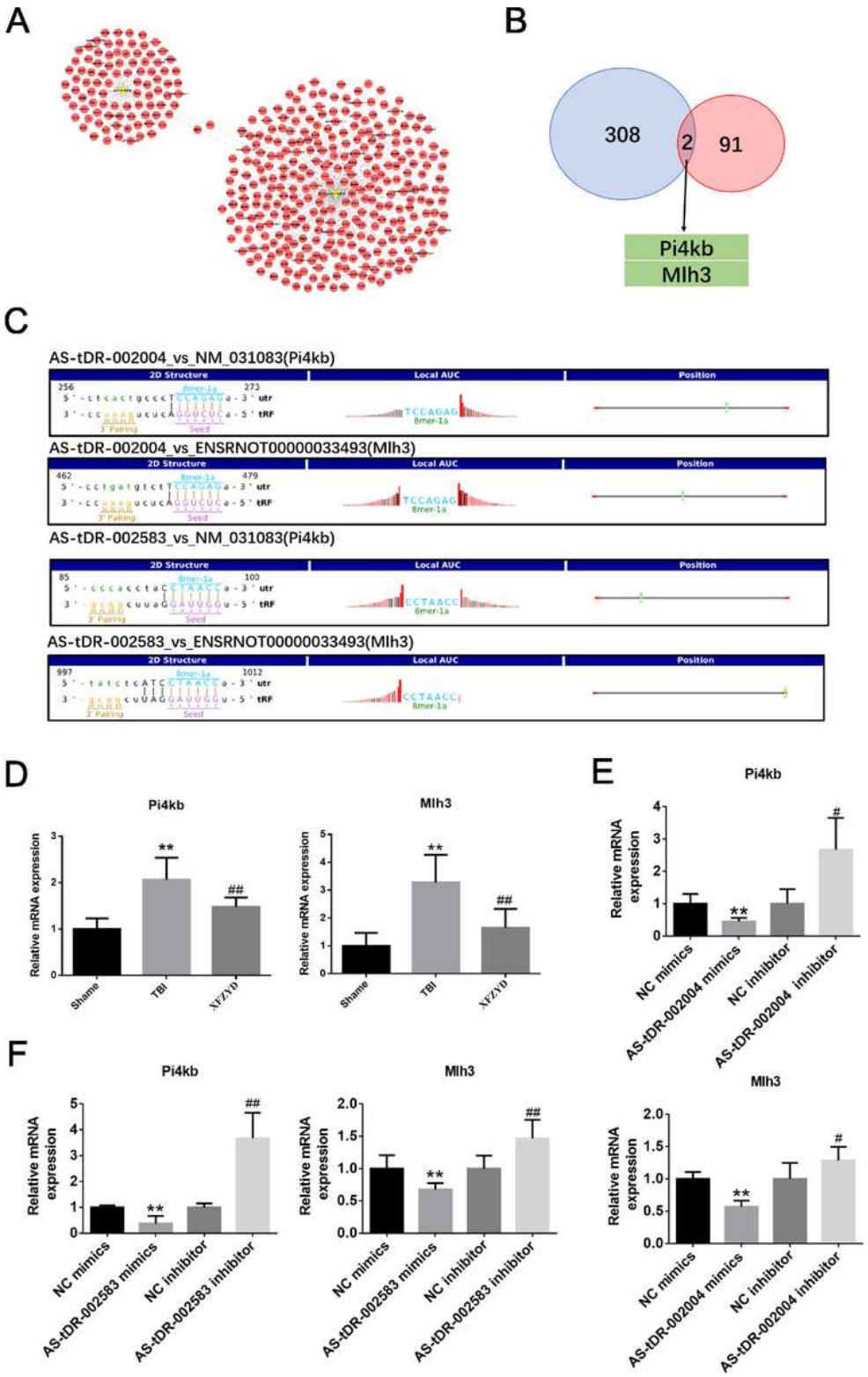

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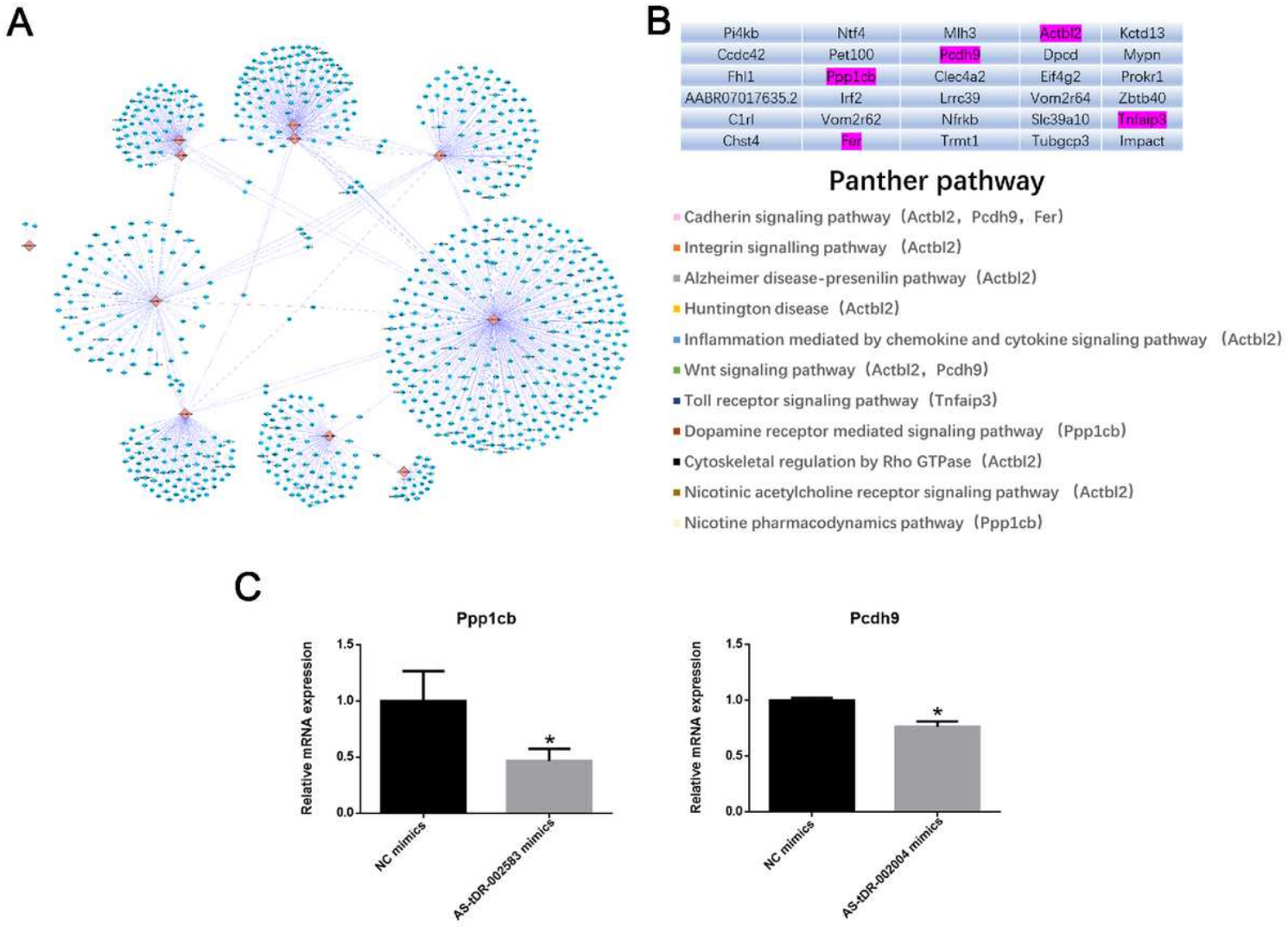

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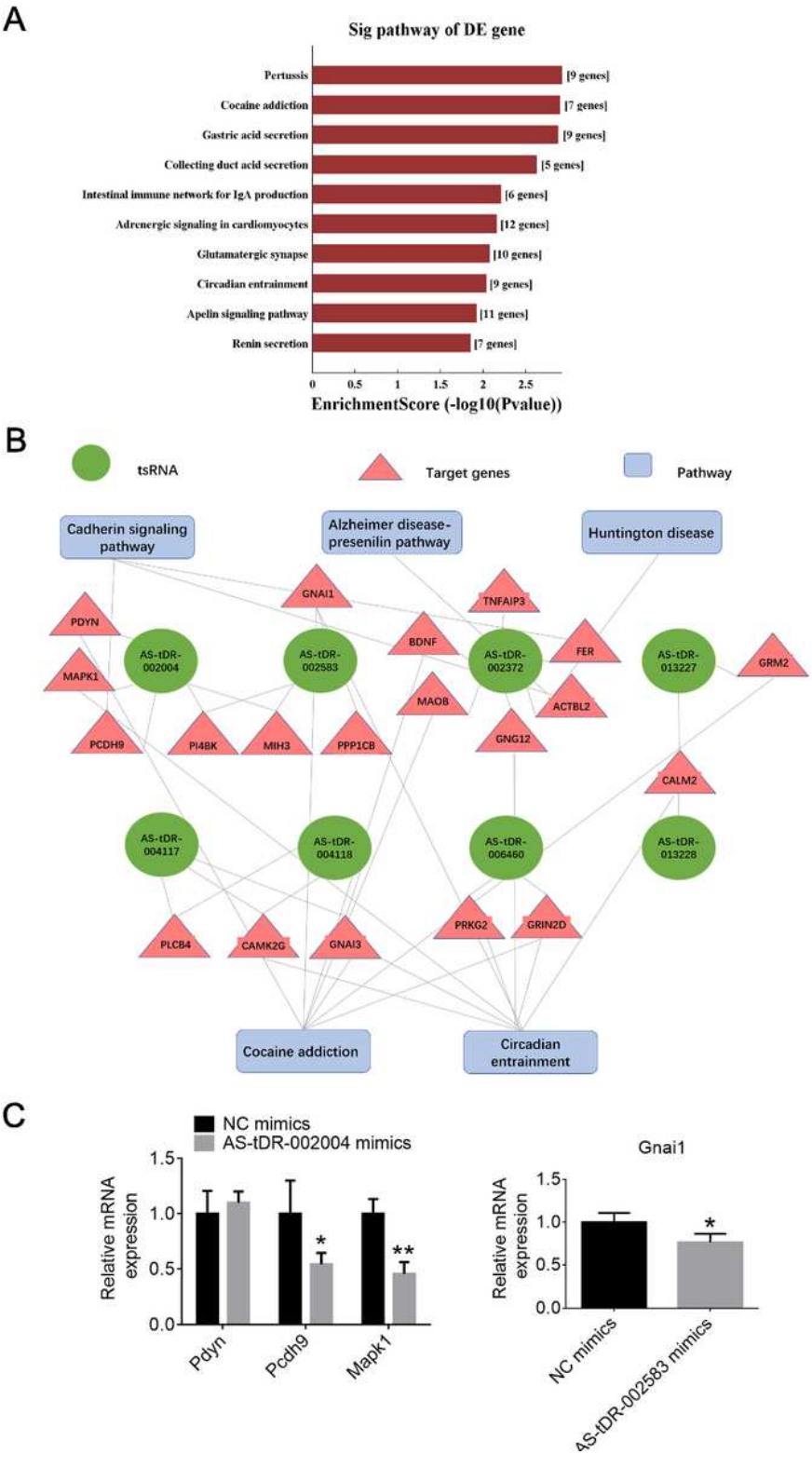

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Supplementary Files

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