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Research

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Comparative analysis of the transcriptomes and small RNAome in Shizhu ginseng and Yuan ginseng based on high-throughput sequencing

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Abstract

Background: Yuan ginseng and Shizhu ginseng, which belong to *P. ginseng*, are widely used as substitutes for wild ginseng in clinical practice. Clinical practice has proved that the clinical efficacy of Shizhu ginseng is better than Yuan ginseng. However, current research cannot completely explain this phenomenon. Considering that small RNA may be one of the pharmacodynamic substances of *P. ginseng*, it is challenging to investigate differential miRNAs between Shizhu ginseng and Yuan ginseng.

Methods: The transcriptome, small RNAome and degradome of *P. ginseng* were studied by high-throughput sequencing.

Results: A total of 63,875 unigenes and 24,154,579 small RNA clean reads were obtained from the roots of *P. ginseng*. Among 3,216 differentially expressed genes, 1190 genes were up-regulated in Yuan ginseng when compared with Shizhu ginseng. 24 known differential miRNAs and 7 novel differential miRNAs were obtained. The 304 targets of 24 differentially expressed miRNA (17 known and 7 novel) families are mainly related to energy metabolism, biotic stress and disease immunity in ginseng itself. 24 known differential miRNAs and 7 novel differential miRNAs were obtained. The 304 targets of 24 differentially expressed miRNA (17 known and 7 novel) families are mainly related to energy metabolism, biotic stress and disease immunity in ginseng itself.

Conclusions: Through the association analysis of mRNA and miRNA, our work gives a better understanding of the difference between Yuan ginseng and Shizhu ginseng. Considering the cross-kingdom regulation of plant miRNAs, our results may provide a foundation for understanding the miRNA-dependent clinical efficacy in *P. ginseng*.

Keywords: Yuan ginseng; Shizhu ginseng; miRNA; Target gene; High-throughput sequencing

Background

Ginseng is the dry root and rhizome of *Panax ginseng* C.A. Mey, which belongs to the *Panax* genus in Araliaceae family. It has widely been used as an important traditional medicine for thousands of years in Eastern Asia, especially China. Evidence from clinical trials demonstrated that *P. ginseng* could play significant roles in many human diseases such as cancer, neurodegenerative diseases (Rajabian et al. 2019) and so on. Owing to its important medicinal properties, *P. ginseng* has been highly valued and has been called “the king of herbs”. Because *P. ginseng* is consumed in large quantities and grows very slowly in natural conditions, wild-grown ginseng cannot meet the growing demand from the medical market. Consequently, *P. ginseng* was artificially cultivated to meet demand for this precious herb and cultivation of this herb dates back at least as far as the Ming dynasty. According to different growth years, cultivated ginseng can be divided into Shizhu ginseng and Yuan ginseng. Yuan ginseng is a ginseng artificially cultivated in gardens or fields for 6 to 8 years. Shizhu ginseng is a ginseng cultivated in a special method for 10 to 20 years, and its medicinal value is comparable to that of wild ginseng.

Although Shizhu ginseng and Yuan ginseng belong to cultivated ginseng, years of medical practice have shown that the clinical efficacy of Shizhu ginseng is significantly better than that of Yuan ginseng. To date, modern pharmacological research has focused on the ginsenosides and polysaccharides, the major bioactive compound of *P. ginseng*. However, it cannot completely explain the reasons for the huge difference in clinical efficacy between Shizhu ginseng and Yuan ginseng.

microRNAs (miRNAs) are a class of endogenous single-stranded RNAs of about 18 nt to 24 nt that can be paired with the 3'-untranslated region (UTR) of a target gene in a complete or incomplete manner. It can regulate gene expression (Dong et al. 2013) at post-transcriptional levels, which plays a major role in lots of aspects (Ying-fang et al. 2019). miRNA research technology such as small RNA sequencing has been widely used in clinical and basic research which has become an important means for scientists to study the function of miRNAs. In this study,

high-throughput sequencing technology was used to compare the differences of miRNAs contained in Shizhu ginseng and Yuan ginseng. In order to explore the mechanism of the difference in clinical efficacy of ginseng with different growth years, we began with the biological functions of non-coding miRNAs contained in Shizhu ginseng and Yuan ginseng.

Methods

Plant material

The 15-year-old Shizhu ginseng and the 6-year-old Yuan ginseng were all collected from the ginseng market of Changbai Mountain in Wanliang Town, Fusong County, Jilin Province, China. They were identified as *Panax ginseng* C.A. Mey by the Associate Professor Hongyan Ma from the School of Traditional Chinese Medicine of Guangdong Pharmaceutical University. The main roots of ginseng were cleaned with distilled water, cut into small pieces, and then immediately frozen in liquid nitrogen until further processing.

RNA extraction

To obtain the RNA used for transcriptome, small RNA and degradome sequencing, the total RNA was extracted by using Trizol reagent according to the manufacturer's protocols, and 100 mg of snap-frozen Shizhu ginseng and Yuan ginseng root tissue was used, respectively. Total RNA integrity was detected by 1% agarose gel electrophoresis. Agilent 2100 Bioanalyzer was used to determine the total RNA concentration and purity of the two ginsengs before additional processing. The criteria for library construction are $RIN \geq 7$ and $28S/18S \geq 0.7$.

High-throughput transcriptome, small RNA and degradome sequencing

Equal amounts of RNA from Shizhu ginseng and Yuan ginseng were separately used to construct transcriptome libraries, small RNA libraries and degradome libraries by reverse transcription and PCR amplification. Agilent 2100 Bioanalyzer was used to evaluate the quality of these libraries. After quality inspection, the libraries were sequenced using the Illumina HiSeq™ 2500 platform by OE biotech company

(Shanghai).

Analysis of transcriptome sequencing data

Bioinformatics analysis was performed on the results of transcriptome sequencing. In order to get high-quality reads, FASTQC was used to control the quality of the raw data. The Trinity software was used to assemble the unigene sets from the clean reads of different year-old roots, using the default parameters. Two samples of non-all-unigene were obtained by short-sequence assembly and splicing, and compared with NCBI non-redundant (NR), EuKaryotic Orthologous Groups (KOG), Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO) and other databases to obtain the protein functional annotation and metabolic pathway information of unigene. The gene expression level for each transcript was calculated by FPKM (reads per kb per million reads). DESeq software was used to identify differentially expressed genes (DEGs). The DEGs was selected using the following criteria: the fold change (FC) > 2 (up-regulation) or the fold change (FC) < 0.5 (down-regulation) and the $p\text{-value} \leq 0.05$. GO functional enrichment and KEGG pathway analyses were performed, respectively, to understand the function of DEGs.

Analysis of small RNA sequencing data

Adapters were removed and low-quantity sequences were filtered from raw data to obtain clean reads. The clean reads were aligned with the Rfam, Gene, Repbase and miRBase 21.0 databases to map known miRNAs, and the unpaired sequences were used for the prediction of novel miRNAs. The expression levels of known miRNAs and newly predicted miRNAs were calculated by TPM (transcript per million). Differentially expressed miRNAs (DEMs) were identified with $FC > 2$ (up-regulation) or $FC < 0.5$ (down-regulation) and the threshold of $p\text{-value} < 0.05$. The $p\text{-value}$ was calculated with Audic Claverie statistic. miRNA targets were predicted from the assembled *P. ginseng* unigene set by the psRNATarget server using default parameters. GO annotation and KEGG pathway analysis were performed on the target genes of DEMs.

Analysis of degradome sequencing data

Clean tags were obtained by data filtering. After comparison with reference genome (unigenes), annotation of Rfam and Genbank database and identification of Poly N, functional annotations for clean tags were obtained. The tags (cDNA-sense) which positively aligned to the reference genome in the clean tags were selected to predict and analyze the miRNA degradation sites. Cleaveland 3 software was used for identification and annotation of miRNA cleavage sites. The reference gene sequence in which the predicted degradation site is located is aligned with the Nr, KEGG, and GO databases to obtain functional annotation information of the degraded gene.

Validation of DEMs and DEGs expression by qRT-PCR

17 DEGs and 9 DEMs of interest were randomly selected for quantitative real-time PCR (qRT-PCR) analysis. The total RNA extracted by using the Trizol reagent was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), and the integrity was evaluated using agarose gel electrophoresis stained with ethidium bromide. Quantification was performed with a two-step reaction process: reverse transcription (RT) and PCR. qRT-PCR analysis was carried out on LightCycler® 480 II Real-time PCR Instrument (Roche, Swiss) using QuantiFast® SYBR® Green PCR Master Mix (Qiagen, Germany). Each sample was run in triplicate for analysis. The expression levels of mRNAs and miRNAs were normalized to 18S rRNA and U6 respectively, and were calculated using the $2^{-\Delta\Delta Ct}$ method.

Results

Total RNA quality, concentration and integrity testing

Agarose gel electrophoresis showed that the 28S and 18S bands of the two samples were clear and complete (Fig. 1), indicating that the extracted RNA was in good integrity. And the total RNA band brightness of the Yuan ginseng was twice as bright as that of the total RNA band of the Shizhu ginseng. This may be due to the long growth period of the Shizhu ginseng that enriched secondary metabolites such as polysaccharide and polyphenols, so the RNA band brightness is weaker than the Yuan

ginseng RNA.

The Agilent 2100 Bioanalyzer was used to perform a quality check on the RNA indicators (Table S1). The concentrations of Shizhu ginseng and Yuan ginseng were 909.24 ng·μL⁻¹ and 1008.79 ng·μL⁻¹, respectively. RNA integrity number (RIN) of Shizhu ginseng and Yuan ginseng was 9.7 and 8.8, respectively. 28S/18S of Shizhu ginseng and Yuan ginseng was 1.9 and 1.8, respectively. The results showed that the total RNA quality met the criteria for high-throughput database construction and the Illumina HiSeqTM 2500 sequencing was performed.

Table S1 The summary of total RNA test run on an Agilent 2100 Bioanalyzer

Sample	concentration (ng·μL ⁻¹)	OD _{260nm} /OD _{280nm}	OD _{260nm} /OD _{230nm}	RIN	28S:18S
Shizhu ginseng	909.24	1.9	2.0	9.7	1.9
Yuan ginseng	1008.79	2.0	2.1	8.8	1.8

The sample meets the requirements for database construction (RIN≥7 and 28S/18S≥0.7), and the total amount can meet the requirements of 2 or more database construction.

DEGs between Shizhu ginseng and Yuan ginseng

To identify transcripts that are involved in different growth year of *P. ginseng*, RNA-seq libraries from Shizhu ginseng and Yuan ginseng were constructed. Approximately 52.5 million raw reads were obtained from each RNA-seq library. After filtering out low-quality reads, clean reads were used for de novo assemble and 63,875 unigenes were obtained as reference genome for follow-up study.

According to the differential gene screening method in Method (FC >2 or FC <0.5, p≤0.05), 3216 differentially expressed genes (DEGs) were found by comparing with the NR database. 1190 genes were up-regulated in the Yuan ginseng compared to those of Shizhu ginseng, while 2026 genes were down-regulated (Fig. 2). To verify DEGs identified by RNA-Seq, 17 DEGs were randomly selected for qRT-PCR verification. The results showed the same expression patterns with RNA-Seq data (Fig. S1).

Functional analysis of DEGs

A gene entry satisfying the condition of p-value ≤ 0.05 and FC > 2 or FC < 0.5 is

defined as GO Term. 1190 up-regulated differentially expressed genes were enriched into 678 GO Terms, and 2016 down-regulated differentially expressed genes were enriched into 1153 GO Terms. Within GO Term analysis, DEGs which were up regulated in the Yuan ginseng mainly related to transcription, DNA-templated, monolayer-surrounded lipid storage body, transcription factor activity, sequence-specific DNA binding, ect. The DEGs which down-regulated in the Yuan ginseng were enriched in the unsaturated fatty acid biosynthetic process, apoplast, heme binding, and so on (Fig. 3).

To further understand the function of DEGs between Shizhu ginseng and Yuan ginseng, we performed KEGG analysis. The Pathway entry enriching the DEG was found Through the Pathway analysis of the DEG. The KEGG Term corresponding to the DEG satisfying $p\text{-value} \leq 0.05$ was set as the significantly enriched KEGG Pathway. Most of the significantly enriched up-regulated DEGs belong to the MAPK signaling pathway, photosynthesis-antenna proteins, spliceosome and so on. Down-regulation of DEGs was significantly enriched in pathways such as phenylpropanoid biosynthesis, fatty acid metabolism, pentose and glucuronate interconversions, ect (Fig. 4).

Identification of miRNAs significantly differentially expressed among Shizhu ginseng and Yuan ginseng

The raw data obtained by high throughput small RNA sequencing was purified to obtain clean reads, and the length distribution of small RNAs with a length of 18-41 nt was statistically analyzed. The small RNA sequences of Shizhu ginseng and Yuanginseng were enriched at the length of 24-21 nt (51.64% and 66.03%, respectively), indicating that miRNA is the main fragment in the deep sequencing results, which provides a powerful basis for the analysis of miRNA.

We performed differential expression analysis between Shizhu ginseng and Yuan ginseng. Differentially expressed miRNAs (DEMs) were selected with fold change >2 or <0.5 and $p\text{-value} \leq 0.05$ by TPM statistic. For known miRNAs, 24 differential miRNAs were obtained, 13 of which were up-regulated and 11 were down-regulated (Fig. 5). For novel miRNAs, a total of 7 differential miRNAs were obtained, of which

5 novel miRNAs were up-regulated in the ginseng and 2 were down-regulated (Fig. 6). 7 DEGs were randomly selected for qRT-PCR verification and the results showed consistence with small RNA sequencing data (Fig. S2).

Target gene prediction and analysis of DEMs

Plant miRNAs show perfect or near-perfect complementarities to their targets. It allows an effective prediction of miRNA targets through computation. To predict the targets of DEMs in *P. ginseng*, we used psRNATarget(Dai et al. 2018), PMRD and TAPIR and other biological software. We identified a total of 304 unigenes from 63,875 assembled *P. ginseng* unigenes to be targets of 17 known miRNA families and 7 novel miRNAs (Table 1).

Table 1 Predicted targets of Panaxginseng miRNAs

miRNA	Target gene
Known-miRNAs	miR159 MYB, ACBP5
	miR160 ARF
	miR162 DCL1, PCL1
	miR164 NAC, FK171, NILP3, FDL3
	miR166i ATHB8, ATB15, REV, MOM1, EK1
	miR172 GATLA, SKP1, APY2, RHF2A, SYLM, PP413
	miR390e TIR1, TMKL1, Y4265
	miR319 NSP2, DET2, TCP4,SR542
	miR393 AFB2, NTPCR, ICE1, TIR1
	miR396 LU7L3, NACK2, TBCD, PPR15, Y3918, GRF4
	miR399 UBC24
	miR4376 ACA8, C78A3, DJC14
	miR4995 GIP, METE
	miR6138 CUL1, SPCS1, RDM4, TMVRN, NCS, Y2913, JAC1, PDAT1, LAZH2, MIRA
	miR6140 APA1, Y5673, LTR3B, TSS, RMP, HSP70, CID9, PP344
	miR6143 XPO7, CSK2B, MLO10
	miR6173 GAF1
	comp17305_c0_seq1 GCP2, VIL2, FK111, AGO7
	comp44202_c0_seq1 RP8L2
Novel miRNAs	comp46638_c0_seq1 SPXM3, P2C16, B561A, ADCK4, AIL5, CSE, ZN363, SKS2
	comp46638_c0_seq1_star M810, ATM, GBF4
	comp48783_c0_seq3 APS3, RH24

comp62834_c0_seq6_star	5NTC, XYLL3, PTR18, KDEL1, GTE4, GDL76, S38A6, TRPC, CBSX6, PLST1
comp72313_c0_seq1	MNS5, BHO79, UGDH5

GO and KEGG functional enrichment analysis was performed on 197 target genes of 17 known miRNA families, and a total of 260 GO functions and 36 pathway pathways were annotated. The GO analysis results contain three parts: biological process, cellular component, and molecular function, and all the most strongly enriched elements are shown in Figure 7A. Within biological process, regulation of transcription, cell differentiation, leaf development and auxin-activated signaling pathway were the most significantly enriched elements; within cellular component, the most significantly enriched element were nucleus, integral component of membrane, and cytoplasm; and within molecular function, binding, catalytic activity, and transcription factor activity groups were the most significantly enriched elements. Through KEGG Pathway functional enrichment analysis, it is known that differential miRNA target genes are enriched in plant hormone signal transduction, Wnt signaling pathway, ubiquitin mediated proteolysis and so on (Fig. 7C).

For target genes of 7 novel miRNAs, we performed KEGG and GO enrichment analysis. A total of 167 GO functional categories and 37 KEGG pathways were annotated. The results of GO analysis have been showed in Figure 7B Within biological process, regulation of transcription, transcription (DNA-templated) and root development were the most significantly enriched elements; within cellular component, the most significantly enriched element were nucleus, integral component of membrane and plasmodesma; and within molecular function, binding, catalytic activity and transcription factor activity groups were the most significantly enriched elements. These GO functional categories of novel miRNA target gene were very similar to those predicted by known differential miRNAs.

KEGG Pathway functional enrichment showed that the target genes of novel miRNAs were enriched in plant hormone signal transduction, p53 signaling pathway, metabolism, ubiquitin mediated proteolysis (Fig. 7D). This was similar to the KEGG

annotation of known miRNA target genes.

miRNA targets validation by degradome sequencing

To further understand the role of miRNA in *P. ginseng*, degradome sequencing of *P. ginseng* was used to identify miRNA targets. The reference gene sequence in which the degraded gene fragment is located is aligned with the NR, KEGG, and GO databases using Cleaveland 3 software to perform functional annotation of the target gene. Two target genes for miR159 were identified, namely GAM1_ORYSI and TCP24_ARATH, which gene functions were GAMYB transcription factor (an important transcription factor in the gibberellin signal transduction pathway) and TCP transcription factor (involved in various signal transduction pathways, connected growth and mediated stress response), respectively. miR393 targets AFB2_ARATH and TIR1_ARATH. It could altering the auxin distribution in plants by negative regulation of TIR1 (transport inhibitor response 1) and F-BOX auxin receptors. This was basically consistent with the target gene type in high throughput sequencing of small RNA. MiR396 participates in the growth process of ginseng by targeting the expression of GRF (transport inhibitor response 1) and protein auxin signaling (F-BOX 3). The target genes of the three novel miRNAs were detected to be associated with SPX factor and TIR1 disease resistance factor (Table 2).

Table 2 The annotation of miRNAs in degradation sequencing

miRNA	Target	Target gene annotation
miR159/319	GAM1_ORYSI	transcription factor GAMYB
	TCP24_ARATH	TCP transcription factor
miR160	ARFR_ORYSJ	auxin response factor
miR164	NC100_ARATH	NAC domain-containing protein
miR165/166	ATB15_ARATH	DNA binding protein
miR172	AP2_ARATH	ethylene-responsive transcription factor
	RH46_ARATH	DEAD-box ATP-dependent RNA helicase
miR393	AFB2_ARATH	protein auxin signaling F-BOX
	TIR1_ARATH	transport inhibitor response 1
miR396	GRF6_ORYSJ	uncharacterized protein
	GRF3_ORYSJ	growth-regulating factor 3

miR399	UBC24_ARATH	ubiquitin-conjugating enzyme E2
miR4376	ACA8_ARATH	autoinhibited calcium ATPase
comp46638	SPXM3_ARATH	SPX domain-containing membrane protein
comp62834	SVP_ARATH	MADS-box protein SVP
comp65451	TMVRN_NICGU	tir-nbs-lrr resistance protein

Discussion

Regulation of plant miRNAs

Plants are rich in miRNA, which can regulate plant growth and development, nutrient metabolism and stress response. For example, the target gene ICE1 (transcription factor ICE1) of miR393 plays a role in cold tolerance by controlling cold-responsive genes(Ohta et al. 2018).

The regulation of some miRNA target genes is involved in the production process of plant secondary metabolites, which is conducive to the accumulation of pharmacodynamic substances. The target gene of miR159 is MYB (Transcription factor MYB51), and the MYB transcription factor is involved in the biosynthesis of fat and glucosinolate(Frerigmann and Gigolashvili 2014). Glucosinolate is a biological compound with anti-cancer and anti-oxidant activity, and its hydrolysis product isothiocyanate can prevent and treat various cancers, scavenge free radicals, protect cardiovascular and cerebrovascular, and delay aging(Konic-Ristic et al. 2016). The target gene of miR172 encodes the glucosyltransferase (GATLA), which is a member of a multi-gene family of plants that transfer single or multiple activated sugars to a range of plant molecules, resulting in glycosylation of plant compounds. The UDP glucosyltransferase gene *P. ginseng* is a key enzyme for the synthesis of ginsenoside(Lu et al. 2017), which is most likely to participate in triterpenoid synthesis(Fan et al. 2018; Khorolragchaa et al. 2014).

Cross-kingdom regulation of plant miRNAs

miRNA target genes are evolutionarily conserved among many known species. It was reported that plant miRNAs could transfer and regulate gene expression in a cross-kingdom manner, namely, affecting the organism from which they do not

originate. Zhang Chenyu et al (Zhang et al. 2012) fed fresh rice which contained abundant MIR168a to mice and found that MIR168a is highly enriched in the serum. MIR168a could bind to the exon 4 of low density lipoprotein receptor adapter protein 1 (LDLRAP1) mRNA and reduces its protein expression in vitro and in vivo, and consequently regulate the lipid metabolism of the mouse. It indicated that the exogenous plant miRNA in food may regulate the expression of target genes in mammals, which laid a foundation for the study of cross-kingdom gene expression regulation by plant miRNAs. Zhang Chenyu et al (Zhou et al. 2015) found that miR2911 contained in honeysuckle significantly inhibited H1N1-encoded PB2 and NS1 protein expression, and consequently played a direct role in the influenza virus in mice.

It has been found that the miRNA of dodder (*Cuscuta* spp.) can as a causative agent to target host mRNA across species to regulate its gene expression and play a role in the process of parasitization (Shahid et al. 2018). Plant miRNAs which were more enriched in beebread than inroyal jelly can prevent larval differentiation into queens and induce development into worker bees by delaying development and decreasing body and ovary size in honeybees (Zhu et al. 2017). It further provides a basis for the cross-kingdom regulation of plant miRNAs.

Mammalian extracellular vesicles (EVs) are a general term for vesicle structures with various membrane structures released by cells. The Roger W. Innes team found that EVs can be involved in cell-to-cell communication, and sRNAs, including miRNAs, can also be transported into them (Baldrich et al. 2019). This provided a transport basis for the possibility of miRNA cross-kingdom regulation.

Wang Yingfang et al (Wang et al. 2018; Wenjuan et al. 2017) detected abundant miRNAs in fresh ginseng and ginseng decoction. It is speculated that ginseng miRNA may be one of the material basis for its clinical efficacy. The difference in clinical efficacy of ginseng with different growth years may be caused by the regulation of its intrinsic miRNA. Based on this, the research group carried out this study. The miRNA differences of Shizhu ginseng and Yuan ginseng with different growth years were compared by high-throughput sequencing. Shizhu ginseng and Yuan ginseng are

different varieties of Chinese ginseng, and their growth years differ by more than 10 years.

Analysis of ginseng miRNA and its target genes

The differential miRNAs of Shizhu ginseng and Yuan ginseng were identified and some related target genes were verified.

In the experiment, there were 17 known miRNAs and 7 novel miRNAs with different in Shizhu ginseng and Yuan ginseng. GO and KEGG functional enrichment analysis was performed on targets of known and novel miRNA families. For GO functional enrichment, in the main category of “biological processes”, the majority of target genes were assigned to terms “regulation of transcription”, “cell differentiation”, and “auxin-activated signaling pathway”. With respect to the main category of “cell component” class, the “nucleus”, “integral component of membrane” and “cytoplasm” was the domain term. Under the third main category of “molecular function”, the dominant terms were “binding”, “catalytic activity”, and “transcription factor activity”. In functional KEGG analysis, target genes are enriched in plant hormone signal transduction, p53 signaling pathway, metabolism, and Ubiquitin mediated proteolysis.

Among them, the target genes of miR396 are LU7L3 (luc7-like protein 3 isoform X2) and TBCD (tubulin-folding cofactor D). LUC7L is defined at the centromeric border of the human alpha-globin domain and inhibits replication of hepatitis B virus by inhibiting enhancer II or basal core promoter activity(Li et al. 2016). TBCD encodes tubulin folding cofactor D, a dynamic cytoskeletal component that coordinates and supports a variety of neuronal processes, including cell division, migration, intracellular trafficking, and signal transduction. Mutations or altered microtubule dynamics can cause neurodevelopmental disorders and neurodegenerative diseases (Flex et al. 2016; Miyake et al. 2016). Studies have shown that TBCD interacts physically with the intracellular domain of Down's syndrome cell adhesion molecule (Dscam), which is important for neural development (Okumura et al. 2015).

The novel miRNA comp46638 targets B561A (cytochrome b561, DM13 and

DOMON domain-containing protein). B561A is found in various organs and cell types of plants and animals. Physiological functions of CYB561s include stress defense, cell wall modification, iron metabolism, tumor suppression, and various neural processes and memory retention(Asard et al. 2013). TBCD and B561A are involved in the process of neurodevelopment, which may be the target of ginseng miRNAs to play a stimulating role.

The novel miRNA comp65451 target gene is involved in the regulation of TIR NBS-LRR (Toll-interleukin-like receptor, nucleotide-binding site and C-terminal leucine-rich repeat), and may be a novel miRNA specific for ginseng species.

The target of miR319 is SR542, which is highly conserved and exists in bacteria and mammals. Autosomal dominant mutations in SRP54 are key factors in co-translational protein targeting pathways, leading to neutropenia like Shwachman-Diamond syndrome (Carapito et al. 2017).

The target of miR393, NTPCR, encodes a cancer-associated nucleoside phosphatase, a cancer-associated PRUNE2 protein that is nucleotide-associated and highly expressed in mature neural tissue, and its neural tissue-specific and post-developmental expression may help maintain maturity the nervous system(Iwama et al. 2011).

Some target genes indirectly exert anti-tumor effects, such as NTPCR, MYB, FDL3, etc., by proteins encoding cancer-associated proteins and secondary metabolites with anticancer effects. Chin AR et al found that oral miR159 mimics significantly inhibited the growth of mouse xenograft breast tumors (Chin et al. 2016).

Conclsions

The above analysis shows that this study lays a foundation for further exploring the differences between Shizhu ginseng and Yuan ginseng and revealing the possible regulation of ginseng miRNA and its target genes. Chinese medicine has incomparable advantages in the treatment of many diseases. However, at present, the mechanism of action of many traditional Chinese medicines is not clear and the effective substances are vague. The research on miRNA and its target genes in traditional Chinese medicine will help to promote the development of traditional Chinese medicine theory and the

integration of traditional Chinese and western medicine. It can be inferred that Chinese traditional medicine miRNA is one of the new mechanisms for Chinese traditional medicine to exert its efficacy, which opens a new field for the exploration of the mechanism of action of Chinese traditional medicine miRNA.

Fig. 1 RNA electrophoresis. M: 1000 marker; Lane 1: total RNA of Shizhu ginseng; Lane 2: total RNA of Yuan ginseng.

Fig. 2 Volcano map of differential gene. The X-axis is the average of all sample expressions used for comparison after normalization, and the Y-axis is log₂ fold change. Red is marked with a difference of significant p-value <0.05 unigene.

Fig. 3 The GO functional analysis of differential expressed genes. A: The GO functional analysis of up-regulated genes. B: The GO functional analysis of down-regulated genes.

Fig. 4 The KEGG analysis of differential expressed genes. A: The KEGG analysis of up-regulated genes. B: The KEGG analysis of down-regulated genes.

Fig. 5 The relative expression level of known diff miRNAs. A: The relative expression level of up-regulated miRNAs in Yuan ginseng. B: The relative expression level of down-regulated miRNAs.

Fig. 6 Differential expression of novel miRNAs.

Fig. 7 GO and KEGG enrichment of target genes of DEMs. A: GO enrichment analysis of target genes of known DEMs. B: GO enrichment analysis of target genes of novel DEMs. C: KEGG enrichment analysis of target genes of known DEMs. D: KEGG enrichment analysis of target genes of novel DEMs.

Supporting information

Fig. S1 qRT-PCR validation of 17 differentially expressed genes. 1: trehalose-6-phosphate synthase; 2: transcription factor TCP15-like; 3: probable ubiquitin-conjugating enzyme E2 24; 4: td1TL1 protein, partial; 5: TSPO(outer membrane tryptophan-rich sensory protein)-related; 6: Cell differentiation protein RCD1 like; 7: growth-regulating factor 4-like; 8: phosphoenolpyruvate/phosphate translocator; 9: auxin-induced protein 22D-like; 10: UGT12; 11: pleiotropic drug resistance transporter; 12: Pyridoxal phosphate (PLP)-dependent transferases superfamily protein; 13: CYP3; 14: class 1 chitinase; 15: pathogenesis-related leaf protein 6-like; 16: endoribonuclease Dicer homolog 1; 17: SPX domain-containing membrane protein

Fig. S2 qRT-PCR validation of differential miRNA.

Abbreviations

miRNA: microRNA; UTR :untranslated region; SRA: sequence read archive; NCBI: National Center for Biotechnology Information; NR: NCBI non-redundant; KOG: EuKaryotic Orthologous Groups; KEGG: Kyoto Encyclopedia of Genes and Genomes; GO: Gene Ontology; FPKM: reads per kb per million reads; DEGs: differentially expressed genes; FC: fold change; TPM: transcript per million; DEMs: differentially expressed miRNAs; qRT-PCR: quantitative real-time PCR; RIN: RNA integrity number; TIR1: transport inhibitor response 1; GRF: transport inhibitor response 1; GATLA: glycosyltransferase; LDLRAP1: low density lipoprotein receptor adapter protein 1; EVs: extracellular vesicles; LU7L3: luc7-like protein 3 isoform X2; TBCD: tubulin-folding cofactor D; Dscam: Down's syndrome cell adhesion molecule; B561A: cytochrome b561, DM13 and DOMON domain-containing protein; TIR NBS-LRR: Toll-interleukin-like receptor, nucleotide-binding site and C-terminal leucine-rich repeat.

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Authors' contributions

YW, ZY, YZ and BH contributed to the study conception and design. Material preparation, data collection and analysis were performed by MP, YC, WW, ZH, WD, ZL, MG, and YY. The first draft of the manuscript was written by MP and YW and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript is approved by all authors for publication.

Competing interests

Conflict of interest The authors declare that they have no conflict of interest.

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Figures

Figure 1

RNA electrophoresis. M: 1000 marker; Lane 1: total RNA of Shizhu ginseng; Lane 2: total RNA of Yuan ginseng.

Figure 2

Volcano map of differential gene. The X-axis is the average of all sample expressions used for comparison after normalization, and the Y-axis is log2 fold change. Red is marked with a difference of significant p-value <0.05 unigene.

Figure 3

The GO functional analysis of differential expressed genes. A: The GO functional analysis of up-regulated genes. B: The GO functional analysis of down-regulated genes.

Figure 4

The KEGG analysis of differential expressed genes. A: The KEGG analysis of up-regulated genes. B: The KEGG analysis of down-regulated genes.

Figure 5

The relative expression level of known diff miRNAs. A: The relative expression level of up-regulated miRNAs in Yuan ginseng. B: The relative expression level of down-regulated miRNAs.

Figure 6

Differential expression of novel miRNAs.

Figure 7

GO and KEGG enrichment of target genes of DEMs. A: GO enrichment analysis of target genes of known DEMs. B: GO enrichment analysis of target genes of novel DEMs. C: KEGG enrichment analysis of target genes of known DEMs. D: KEGG enrichment analysis of target genes of novel DEMs.

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