Analysis of the miRNA expression profile of laboratory red crucian carp under low-dose caesium-137 irradiation

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Research Article

Keywords: Laboratory red crucian carp, irradiation, microRNA

Posted Date: March 10th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1222230/v1

Abstract

Radiation can cause the differential expression of biological miRNA molecules, but research on miRNAs and biological radiation damage is insufficient. This research was based on the development of the experimental animal resource laboratory red crucian carp (LRCC) to explore the feasibility of its application in the detection of low-dose ionizing radiation-induced biological damage in aquatic environments and the development of related molecular markers. Adult LRCC were irradiated with caesium-137 at 0.3 Gy, while RNA-seq and bioinformatics techniques were used to identify miRNAs that were differentially expressed relative to their levels in the nonirradiation group. Analysis of liver sections showed that liver cells in the radiation group showed nuclear pyknosis. In this study, 34 miRNAs differentially expressed in the liver of LRCC after irradiation were identified, among which seven were new crucian carp miRNAs; the prediction analysis of their target genes showed that a total of 632 target genes were predicted. The results of comprehensive GO enrichment and KEGG pathway analyses showed that these target genes were mainly involved in energy transfer and material catabolism, especially malonyl-CoA biosynthesis, acetyl-CoA carboxylase activity, fatty acid biosynthesis and metabolism, and pyruvate metabolism; in addition, the AMPK signalling pathway was the most active pathway. This study shows that LRCC have a certain sensitivity to radiation, and the miRNAs identified may be used as preselected biomarkers for radiation damage detection.

Introduction

Nuclear reactions and nuclear leaks release radioactive elements, including caesium-137, which emits nuclear gamma rays that penetrate surrounding objects. Nuclear ionizing radiation is harmful to most living things. According to the radiation dose effect on the human body, exposure to 0.2 Gy of nuclear radiation is safe, but potential long-term radiation exposure still has a cumulative dose-dependent hazard effect in that if the radiation dose exceeds 0.2 Gy, haematological changes and organ damage gradually occur; short-term exposure to radiation doses exceeding 4 Gy may cause acute death. With the continuous development of nuclear science and radiation protection technology, the possibility of ultrahigh-dose nuclear radiation injury is relatively low, but low-dose nuclear or ionizing radiation events are still possible. Radiation medicine has established protocols for emergency treatment after radiation, and biomarkers are needed to evaluate the individual dose of irradiation to determine its impact on health.

MicroRNAs (miRNAs) are important regulators of gene expression after transcription. They play a very important role in the growth and development of organisms, metabolic pathways of organisms, and resistance to biotic or abiotic stresses. Studies have shown that miRNAs are involved in the biological response to radiation and are closely related to radiation sensitivity and radiation bystander effects. The radiation-induced changes in the expression of miRNAs also depend on factors such as the specific cell, dose, and exposure time(El Bezawy et al., 2019; Hu et al., 2011; Troschel et al., 2018), and it is very possible to use these changes as an biological radiation dosimeter. Sangsu Shin irradiated A549 cells with 20 Gy and 40 Gy caesium-137 and found that 4 miRNAs were downregulated after 20 Gy irradiation and that 2 miRNAs were downregulated and 8 miRNAs were upregulated after 40 Gy irradiation (Shin et al., 2009). Florczyk M exposed whitefish (*Coregonus lavaretus*) to microcystins and studied miR-122-5p as a plasma biomarker of liver damage in fish(Florczyk et al., 2016). Therefore, miRNAs should also be used as biomarkers for detecting biological radiation-induced damage.

Laboratory red crucian carp (LRCC) was domesticated from wild red crucian carp. Our research team used induction of natural reproduction in captivity and artificial gynogenesis techniques to separately cultivate a closed colony (HN:CARV1) and an inbred strain (C1HD) of LRCC(Wu, 2016). In this study, LRCC was used as the research object, and a low-dose (0.3 Gy) caesium-137 irradiation group and a nonirradiation group were used to analyse the miRNA expression profile in the liver. RNA-seq and bioinformatics analysis techniques were combined to identify differentially expressed miRNAs that are sensitive to nuclear radiation and to predict their target genes and analyse their functions. This research was based on the development of the experimental animal resource LRCC. First, it investigated the sensitivity of LRCC to irradiation and explored the feasibility of its application in the detection of low-dose ionizing radiation-induced biological damage in aquatic environments. In addition, it could be used to develop miRNAs as molecular markers for detecting biological radiation-induced damage.

Materials And Methods

Ethics statement

The procedures were conducted in accordance with the approved guidelines. Individual experimental fish were housed in an aquarium (0.3 m^3) with a suitable pH (7.0-8.0), water temperature (21-24°C), and dissolved oxygen content (6.0-8.0 mg/L) and adequate forage at the University of South China, Department of Laboratory Animal Science, China.

Experimental fish and treatments

Laboratory red crucian carp (CIHD strain, 6 tails, 1 year old, body length (9.36±0.65) cm, body weight (32.35±5.41) g) were bred and provided by the Department of Laboratory Animal Science, University of South China. After the LRCC were domesticated in an aquarium for 7 days, they were randomly divided into the irradiation group (radiation group) and the nonirradiation group (control group). Fish in the irradiation group were treated with a single fraction of whole-body irradiation with 0.3 Gy caesium-137 in a biological irradiator (Nuclear Power Institute of China, HXFS-IA, China), with a dose rate of 0.5 cGy/s and an irradiation time of 60 s; fish in the control group were not treated with radiation. Twenty-four hours after irradiation, the experimental fish were anaesthetized with 60 mg/L MS-222 (Sigma–Aldrich, St. Louis, MO, USA), and the livers of fish in the two groups were harvested. First, the livers were cleaned with RNase-free water (Sangon, B541018-0010, China) and immediately placed in liquid nitrogen for quick freezing. Then, the total RNA of the livers was extracted with an RNA extraction kit (OMEGA, R6934, USA). Finally, the total RNA concentration was determined by NanoDrop spectrophotometry (Thermo Scientific, USA) and formaldehyde denaturing gel electrophoresis.

Paraffin sectioning of liver tissue

The harvested livers were directly transferred to Bouin's solution for fixation. After fixation for 48 hours, they were dehydrated through an alcohol gradient, cleared in xylene and embedded in paraffin. Then, the sections were sliced with a Leica microtome (Leica, RM2235, Germany), and the slice thickness was set to 5 μm. After H&E staining and sealing with neutral gum, the sections were visualized with a high-power microscope (Olympus, BX63F, Japan), and images of the sections were acquired.

Construction and sequencing of the miRNA library

Small RNAs with a length of 15-41 nt were separated from total RNA using 15% TBE-urea polyacrylamide gel electrophoresis, and adapters were ligated to their 5' and 3' ends. Then, RNA was reverse transcribed into cDNA and amplified by PCR. An Agilent 2100 Bioanalyzer (Agilent, G2938A, USA) was used to confirm the quality and length of the library, and the library was sequenced on the HiSeq X Ten platform (Illumina, PE150, USA). The construction and sequencing of the miRNA library were carried out at Shanghai OE Biotech Co., Ltd.

Sequence analysis and target miRNA screening

The original data generated by sequencing were processed by removing the adapter sequences, low-quality sequence and the contaminating sequences to obtain clean reads. Clean reads of 15-41 nt were selected, and the crucian carp genomic library (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/003/368/295/GCF_003368295.1_ASM336829v1/GCF_003368295.1_ASM336829v1_genomic), crucian carp gene library (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/003/368/295/GCF_003368295.1_ASM336829v1/GCF_003368295.1_ASM336829v1_rna), Rfam library (version 10.0) (Griffiths-Jones et al., 2003), cDNA sequence library, and species repeated sequence library(Chen, 2004) were searched by BLAST to annotate sRNAs. Then, Bowtie software (Langmead, 2010) was used to filter out tRNAs, rRNAs, piRNAs, and snRNAs. By comparison with a miRNA database (http://www.mirbase.org/textsearch.shtml?q=Teleostei), the known miRNAs were analysed, and new miRNAs were predicted. Then, miRNA read counts were normalized as TPM (transcripts per million) values. According to the miRNA expression analysis, miRNAs with significant differential expression between the control group and the radiation group were identified.

q-PCR verification of differentially expressed miRNA

Three differentially expressed miRNAs were randomly selected, and Primer Premier 5.0 software was used to design primers (Table 1), which were synthesized by Sangon Biotech Co., Ltd. According to the operating instructions of a miRNA first-strand cDNA synthesis kit (Sangon, B532451, China) and a q-PCR kit (Thermo Fisher, 00775499, USA), RNA was reverse transcribed to obtain cDNA, and q-PCR was performed. There were 6 samples in total between the two groups, and each sample was analysed in triplicate. q-PCR was performed in an ABI 7500 Real-Time PCR System, and the thermal cycling program used for amplification reaction was as follows: predenaturation at 95°C for 10 min followed by 45 cycles at 95°C for 10 s, annealing at 60°C for 30 s, and extension at 72°C for 15 s. A final extension step was performed at 72°C for 5 min, and the amplification products were stored at 4°C. U6 was used as the internal reference gene for miRNA expression.

Table 1
q-PCR primer sequences and annealing temperatures

miRNA ID	Primer sequence (5'-3')	Primer length (bp)	Tm (°C)
ccr-miR-203b-3p	GGCGTGAAATGTTCAGGACCACTTG	25	60
dre-miR-24b-3p	TGGCTCAGTTCAGCAGGAACC	21	60
novel-74-mature	AACACGGCAGGTGACTGGTC	20	60

Target gene prediction and functional analysis

To identify the targets of differentially expressed miRNAs, miRanda (Creighton et al., 2008) was used to predict their target genes. GO (Gene Ontology) enrichment and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses were used to annotate and summarize the functions of the target genes. In GO and KEGG analyses, GO terms and KEGG pathways with $P<0.05$ were defined as significantly enriched.

Results

Visualization of liver tissue sections

Before irradiation, fish in the radiation group had no radiation-induced body surface damage and no abnormalities in behaviour relative to fish in the control group. Microscopic observation of liver sections showed that the liver cells of fish in the radiation group had nuclear pyknosis (Fig. 1).

miRNA sequencing

The length and copy number data of the clean read sequences generated through deep sequencing showed that most of them were concentrated in the range of 21~23 nt and that most were 22 nt long; these sequences accounted for more than 40% of the total reads (Fig. 2). sRNAs were annotated and filtered by comparing the clean reads with data in multiple databases. The mapping rate of the clean reads to sequences in the crucian carp genome was greater than 96% (Table 2). By comparison with *Teleostei* library in the miRBase database (version 22.0), a total of 789 known miRNAs and 995 newly predicted miRNAs were identified from the 6 samples (Table 3).

Table 2
Classification annotation and gene mapping rate of each sample read

Sample	Annotation type	Reads	Percentage of total (%)	Total reads	Aligned reads	Aligned (%)
C1	rRNA	1533	0.01	16144231	15682811	97.14
	tRNA	210	0			
	snRNA	1404	0.01			
	Cis-reg	699	0			
	Other RNA	2049	0.01			
	gene	10503	0.07			
	repeat	265885	1.65			
	Known miRNA	10325275	63.96			
	unannotated	5536673	34.3			
C2	rRNA	1031	0.01	15739048	15403156	97.87
	tRNA	87	0			
	snRNA	782	0			
	Cis-reg	370	0			
	Other RNA	557	0			
	gene	4642	0.03			
	repeat	176246	1.12			
	Known miRNA	11000231	69.89			
	unannotated	4555102	28.94			
C3	rRNA	1016	0.01	14167690	13682853	96.58
	tRNA	165	0			
	snRNA	907	0.01			
	Cis-reg	512	0			
	Other RNA	615	0			
	gene	16765	0.12			
	repeat	341553	2.41			
	Known miRNA	8793238	62.07			
	unannotated	5012919	35.38			
R1	rRNA	1157	0.01	14716192	14317228	97.29
	tRNA	281	0			
	snRNA	1691	0.01			
	Cis-reg	619	0			
	Other RNA	1138	0.01			
	gene	9951	0.07			
	repeat	324946	2.21			
	Known miRNA	9809742	66.66			
	unannotated	4566667	31.03			
R2	rRNA	947	0.01	15251035	14845007	97.34

Sample	Annotation type	Reads	Percentage of total (%)	Total reads	Aligned reads	Aligned (%)
	tRNA	162	0			
	snRNA	1368	0.01			
	Cis-reg	564	0			
	Other RNA	1126	0.01			
	gene	4281	0.03			
	repeat	174387	1.14			
	Known miRNA	10567717	69.29			
	unannotated	4500483	29.51			
R3	rRNA	970	0.01	14477040	14153426	97.76
	tRNA	122	0			
	snRNA	1147	0.01			
	Cis-reg	605	0			
	Other RNA	698	0			
	gene	5335	0.04			
	repeat	162716	1.12			
	known miRNA	10208710	70.52			
	unannotated	4096737	28.3			

Table 3
Total amount of known miRNAs and newly predicted miRNAs

Sample	Known miRNAs	Novel miRNAs
C1	629	660
C2	608	640
C3	654	657
R1	652	645
R2	637	634
R3	623	636

Differentially expressed miRNAs

According to comparative analysis of miRNA expression between the radiation group and the control group, 34 miRNAs with significant differential expression were identified, among which 16 miRNAs were upregulated and the others were downregulated. Among them, abu-miR-152, abu-miR-19b, ssa-miR-125b-2-3p, dre-miR-18c, dre-miR-126a-3p, gmo-miR-27d-3p, dre-miR- 9-7-3p, gmo-miR-140-5p, etc. had a log2 fold change >2 and $P<0.05$. The expression differences between the radiation group and the control group were more obvious in cluster analysis (Table 4, Fig. 3). Cluster analysis showed that 27 of the mature miRNAs among the differentially expressed miRNAs belonged to 22 miRNA gene families and that the other 7 were newly predicted precursor miRNAs. In the *Teleostei* library of miRBase, the differentially expressed miRNAs were matched to snapper (abu, *Archamia buruensis (Bleeker, 1856)*), medaka (ola, *Oryzias latipes*), Atlantic salmon (ssa, *Salmo salar*).), koi carp (ccr, *Cyprinus carpio haematopterus*), zebrafish (dre, *Danio rerio*), Atlantic cod (gmo, *Gadus morhua*), and channel catfish (ipu, *Ictalurus punctatus*) sequences. Three differentially expressed miRNAs, ccr-miR-203b-3p, dre-miR-24b-3p, and novel-370-mature, were selected for q-PCR verification. The q-PCR results showed that the expression trends of these miRNAs were basically the same as those identified by sequencing. This consistency indicates the credibility of the sequencing results (Fig. 4).

Table 4
Differentially expressed miRNAs

miRNA_id	log2FoldChange	*p*Value	Regulation	Sequence
abu-miR-152	Inf	0.019	Up	TCAGTGCATAACAGAACTTTG
abu-miR-454b	3.32	0.048	Up	TAGTGCAATATTGCTTATAGGG
abu-miR-99b	4.25	0.006	Up	AACCCGTAGATCCGATCTTGT
dre-miR-126a-3p	3.77	0.000	Up	TCGTACCGTGAGTAATAATGC
dre-miR-18c	2.16	0.034	Up	TAAGGTGCATCTTGTGTAGTTA
dre-miR-202-5p	5.49	0.001	Up	TTCCTATGCATATACCTCTTTG
dre-miR-24b-3p	5.71	0.002	Up	TGGCTCAGTTCAGCAGGAACCG
dre-miR-9-7-3p	3.06	0.014	Up	TAAAGCTAGAGAACCGAAAGTA
gmo-miR-29d-3p	2.59	0.030	Up	TAGCACCATTTGAAATCGGT
ipu-miR-181a	1.92	0.039	Up	AACATTCAACGCTGTCGGTGAG
ipu-miR-29a	Inf	0.019	Up	ACTGATTTCCTCTGGTGTT
novel-402-mature	2.95	0.041	Up	AACACTGCTGACATGTTATCTT
novel-74-mature	10.45	0.045	Up	AACACGGCAGGTGACTGGTCA
ola-miR-200b	3.02	0.046	Up	TAATACTGCCTGGTAATGATG
ssa-miR-125b-2-3p	2.79	0.047	Up	CAGGTGAGGTCCTTGGGAAC
ssa-miR-29a-3p	2.80	0.049	Up	TAGCACCATTTGAAATCAGT
abu-miR-19b	-3.33	0.005	Down	TGTGCAAATCCATGCAAAACTG
abu-miR-205	-2.88	0.021	Down	TCCTTCATTCCACCGGAGTCTG
ccr-miR-203b-3p	-2.60	0.036	Down	GTGAAATGTTCAGGACCACTTG
ccr-miR-222	-4.56	0.001	Down	AGCTACATCTGGCTACTGGG
dre-miR-27c-3p	-4.08	0.027	Down	TTCACAGTGGTTAAGTTCTGC
gmo-miR-140-5p	-7.68	0.000	Down	CAGTGGTTTTACCCTATGGTA
gmo-miR-18a-2-5p	-2.96	0.032	Down	TAAGGTGCATCTAGTGCAGAT
gmo-miR-21-3p	-Inf	0.002	Down	CGACAACAGTCTGTAGGCTGT
gmo-miR-27d-3p	-4.22	0.001	Down	TTCACAGTGGCTAAGTTCCG
novel-261-mature	-3.79	0.016	Down	TTCCATGCTGAAATATCTGATT
novel-370-mature	-3.61	0.039	Down	AAAGTGTGGCCTTTCTTGTCT
novel-451-mature	-Inf	0.046	Down	TTTGGCTAGAGGAAATGGTTT
novel-565-mature	-Inf	0.036	Down	TGACGGTGCTGACGTGTTATC
novel-88-star	-Inf	0.002	Down	TTAAAGACGGTTATCTAATCAG
ola-miR-126-3p	-4.20	0.010	Down	TCGTACCGTGAGTAATAATG
ola-miR-140-3p	-4.27	0.014	Down	ACCACAGGGTAGAACCACGG
ola-miR-148	-4.62	0.009	Down	TCAGTGCATTACAGAACTTT
ola-miR-27d-3p	-6.18	0.002	Down	TTCACAGTGGCTAAGTTC

Prediction and functional analysis of target genes of differentially expressed miRNAs

miRanda was used to predict target genes, and a total of 632 target genes were predicted for 16 differentially expressed miRNAs. Among them, the miRNAs with a large number of target genes were abu-miR-205 (90 targets), dre-miR-24b-3p (147 targets), novel -565-mature (59 targets), novel-74-mature (64 targets), ola-miR-140-3p (30 targets) and ssa-miR-125b-2-3p (182 targets).

GO analysis of the target genes showed that the main functions of the target genes were related to biological regulation, cell growth and development, and metabolic processes; they were secondarily related to cell structure processes, such as the formation of cell membranes, the formation of molecular complexes, and the formation of neuronal synapses. The remaining target genes were related to functions of biological macromolecules, mainly catalytic activity, ion channel regulation, enzyme activity regulation, protein binding and receptor recognition (Fig. 5 and Fig. 6). The results of the KEGG analysis showed that the functions of most target genes were related to activities in systems such as the neurological, endocrine and immune systems, mainly in lipid metabolism, amino acid metabolism and carbohydrate metabolism. The rest were involved in signal transduction, transport and catabolism, and cell growth and death (Fig. 7 and Fig. 8). The combined results of GO analysis and KEGG analysis indicated that the target genes were the most active in functions of energy transfer and material catabolism, including mainly malonyl-CoA biosynthesis, acetyl-CoA carboxylase activity, fatty acid biosynthesis and metabolism, pyruvate metabolism, the AMPK signalling pathway, etc.

Discussion

Twenty-five percent Gy is a safe dose and a low dose for humans, but the value of a low irradiation dose for LRCC cannot be completely determined. Previous studies have shown that 1.94 Gy caesium-137 irradiation can cause changes in the levels of SOD, GSH-PX and HSP70, molecules related to oxidative stress, in the liver of LRCC. J. Lemos used 0.1~1.0 Gy X-ray irradiation to stress zebrafish, and the results showed that the offspring's peripheral blood DNA was damaged to different degrees within 24 hours after irradiation (Lemos et al., 2017). We collectively considered the results of research on radiation dose effects related to humans, zebrafish and LRCC and established 0.3 Gy as the low dose of caesium-137 irradiation in this study. This study showed that exposure to 0.3-Gy irradiation stress did not cause visible damage or death in the experimental LRCC. Analysis of liver sections showed that some cells had nuclear pyknosis, and differential expression of miRNAs could still be detected.

The biological effects of low-dose radiation are categorized as excitatory effects and adaptive effects, among which excitatory effects are mainly manifested in promoting growth, prolonging the lifespan, and enhancing immune function (Jargin, 2020). The excitatory effect of radiation, as proposed by Luckey T.D., indicates that the energy of low-dose rate irradiation can be absorbed by the organism and converted into chemical energy that can be used by itself, accelerate metabolism, and even produce certain benefits to the organism(Luckey, 2006; Sagan, 1989). This study also showed that the target genes of differentially expressed miRNAs are involved in substance metabolism, cell damage repair, immune responses, etc. In particular, the metabolism of fats, carbohydrates, amino acids, and pyruvate is enhanced, and the assembly of the nucleus, cytoplasm, and cell membrane is active.

In this study, the irradiation group and nonirradiation group of LRCC were used as the research objects, and 34 differentially expressed miRNAs were identified through RNA-seq and bioinformatics analysis. Among these miRNAs, 10—abu-miR-99b, dre-miR-126a-3p, dre-miR-202-5p, dre-miR-24b-3p, abu-miR-19b, ccr-miR-222, gmo-miR-140-5p, gmo-miR-21-3p, gmo-miR-27d-3p, ola-miR-27d-3p—exhibited extremely significant differential expression, with $P<0.01$. Studies have shown that miR-99 family miRNAs are related to the repair of radiation-induced DNA damage. By targeting the SWI/SNF chromatin remodelling factor SNF2H/SMARCA5, the speed and overall efficiency of DNA damage repair after irradiation can be reduced, which is helpful for fractional radiotherapy(Mueller et al., 2013). The miR-24 family negatively regulates FERMT1, which can enhance the sensitivity of cells to ionizing radiation(Yan et al., 2019). The miR-27 family is related to fat metabolism and lipid cell differentiation and plays an important role in regulating dynamic homeostasis of energy metabolism(Chen et al., 2012; Vickers et al., 2013). Wen-Jun Wei used high-energy iron ions to irradiate the whole body of mice and found that the expression levels of miR-21a and miR-200b in the circulating blood were increased with a strong dose-effect relationship(Malkani et al., 2020). The differentially expressed miRNAs identified in this experiment may be used as preselected biomarkers for the biological effects of irradiation in aquatic animals.

LRCC has many advantages as a model fish and has shown certain applications in environmental monitoring of chemicals, heavy metals and pesticides in aquatic environments and aquatic ecotoxicology research(Mennigen et al., 2017; Yang et al., 2020; Zhou et al., 2019).

The effects of radiation observed in previous research and this study show that LRCC also have a high sensitivity to nuclear radiation. A radiation dose of 0.3 Gy can cause nuclear constriction in hepatocytes and changes in liver miRNA expression; 1.94 Gy can cause changes in blood physiology and biochemistry, enzymology, protein expression and other parameters; and 31 Gy may cause death in 50% of LRCC. Therefore, LRCC may have great research and application importance in evaluating the biological effects of radiation in aquatic environments and in evaluating the safety of radiation, and this fish can be used as a model experimental animal for radiation biology research.

Declarations

Data Availability

All the datasets used and/or analyzed throughout the present study are available from

the corresponding author on reasonable request.

Acknowledgments We would like to sincerely appreciate many researchers who help to complete this manuscript.

Author contributions YD.W, KJ.C, DS.W designed the study, carried out the analyses and prepared and draft the manuscript. YD.W, XL.L, KJ.C, DS.W and ZH. Z performed the technical discussions. YD. W, XL.L and KJ.C participated in data simulation and discussions. YD, W, KJ. C, YX. L and JP. Y were involved in the statistical analysis. All the authors read and approved the final manuscript.

Animal Research (Ethics)

The procedures were conducted in accordance with the approved guidelines. Individual experimental fish were housed in an aquarium (0.3 m^3) with a suitable pH (7.0-8.0), water temperature (21-24 °C), and dissolved oxygen content (6.0-8.0 mg/L) and adequate forage at the University of South China, Department of Laboratory Animal Science, China.

Consent to Publish (Ethics) All authors agree to publish.

Conflict of Interest The authors declare no competing interests.

Plant Reproducibility None

Clinical Trials Registration None

Funding This work was supported by the Natural Science Foundation of Hunan Province (Grant No. 2019JJ40077, 2021JJ40344). Funds for this project provide funding for reagents, consumables, and laboratory apparatus for this study.

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Compliance with ethical standards

Competing interests The authors declare no competing interests.

Ethical approval The fish researchers were certified under a professional training course for laboratory animal practitioners held by the Institute of Experimental Animals, Hunan Province, China (Certificate No. 4263). This study was carried out in accordance with the recommendations of the Administration of Affairs Concerning Experimental Animals for the Science and Technology Bureau of China. The protocol was approved by the Administration of Affairs Concerning Experimental Animals for the Science and Technology Bureau of China.

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Figures

Figure 1

Paraffin section of liver

Note: a. Control group, 400×; b. Radiation group, 400×.

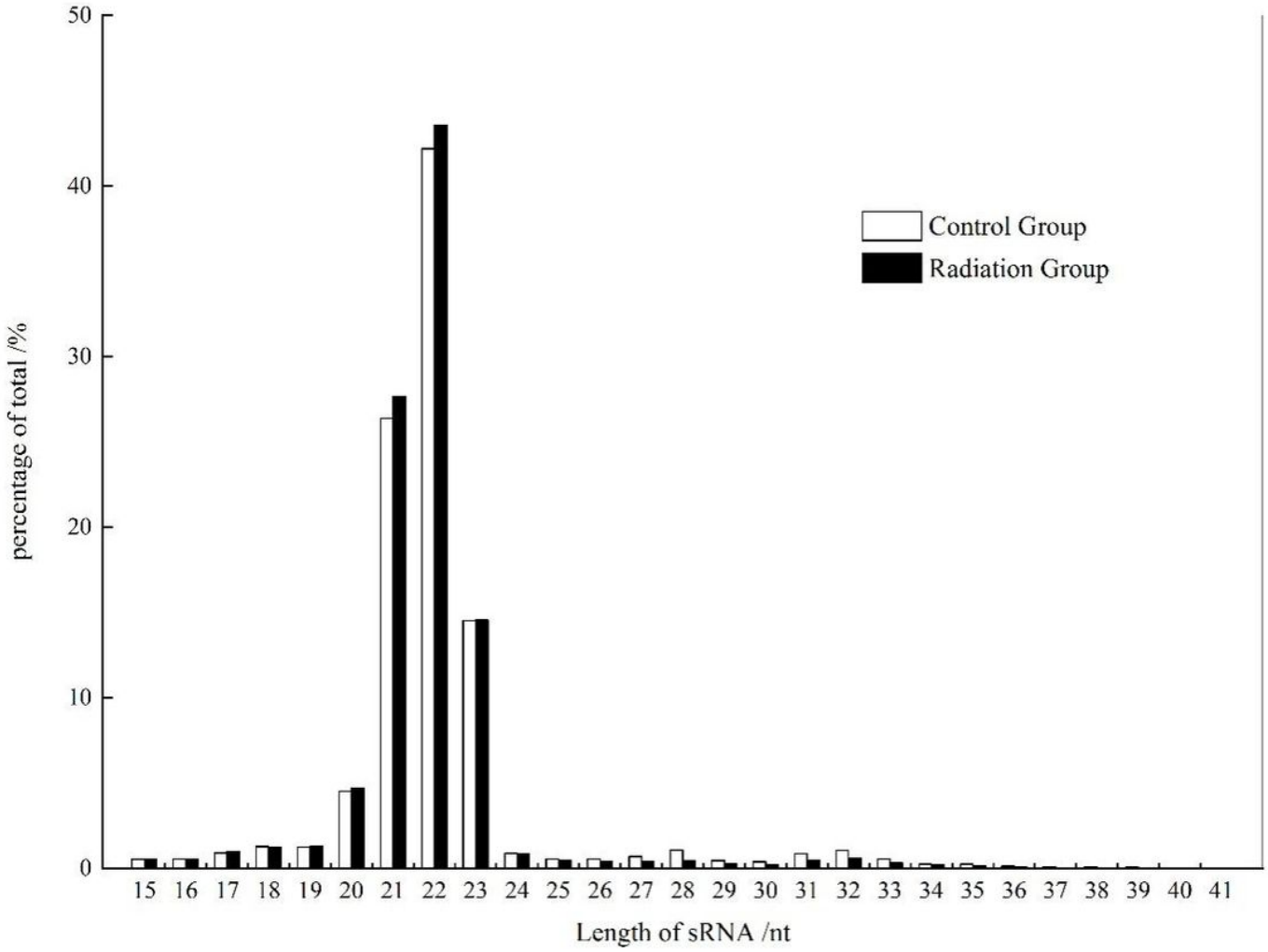

Figure 2

Distribution of sRNA sequence lengths

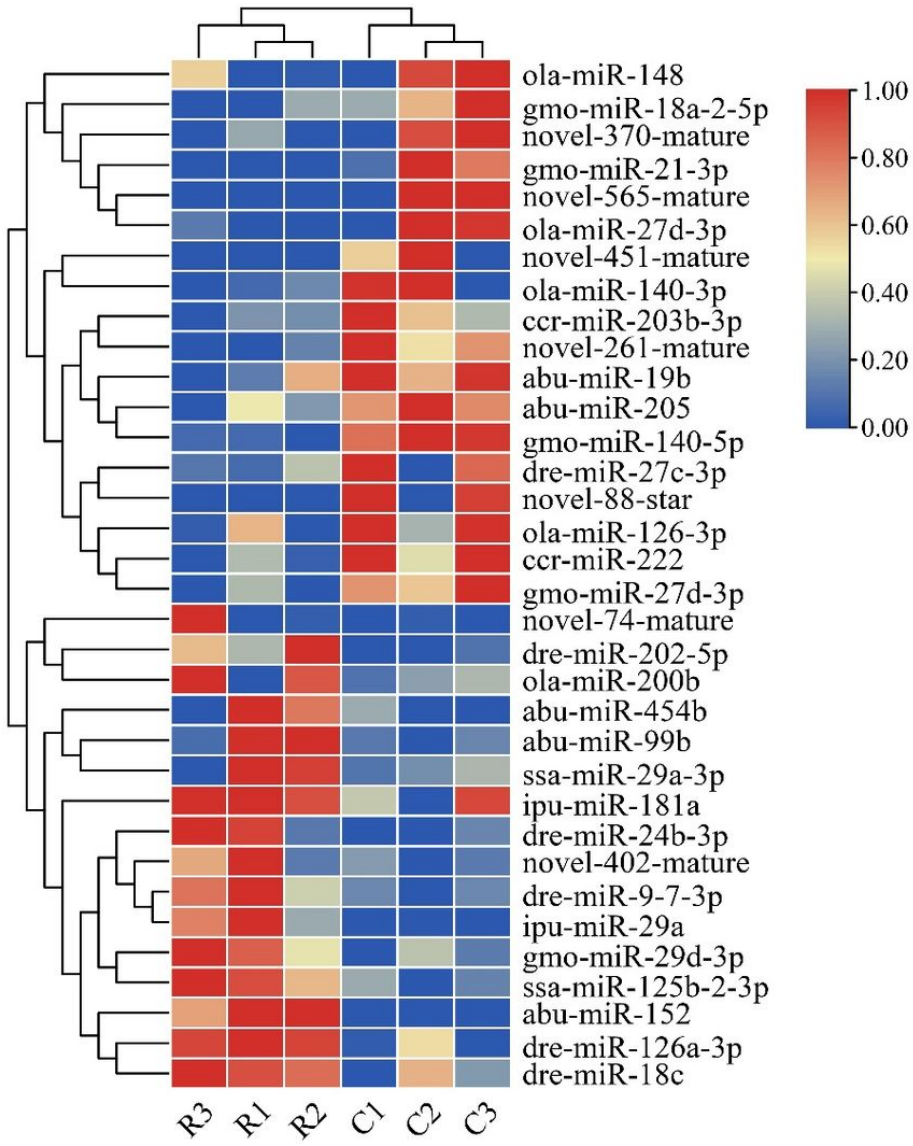

Figure 3

Hierarchical clustering of the differentially expressed miRNAs

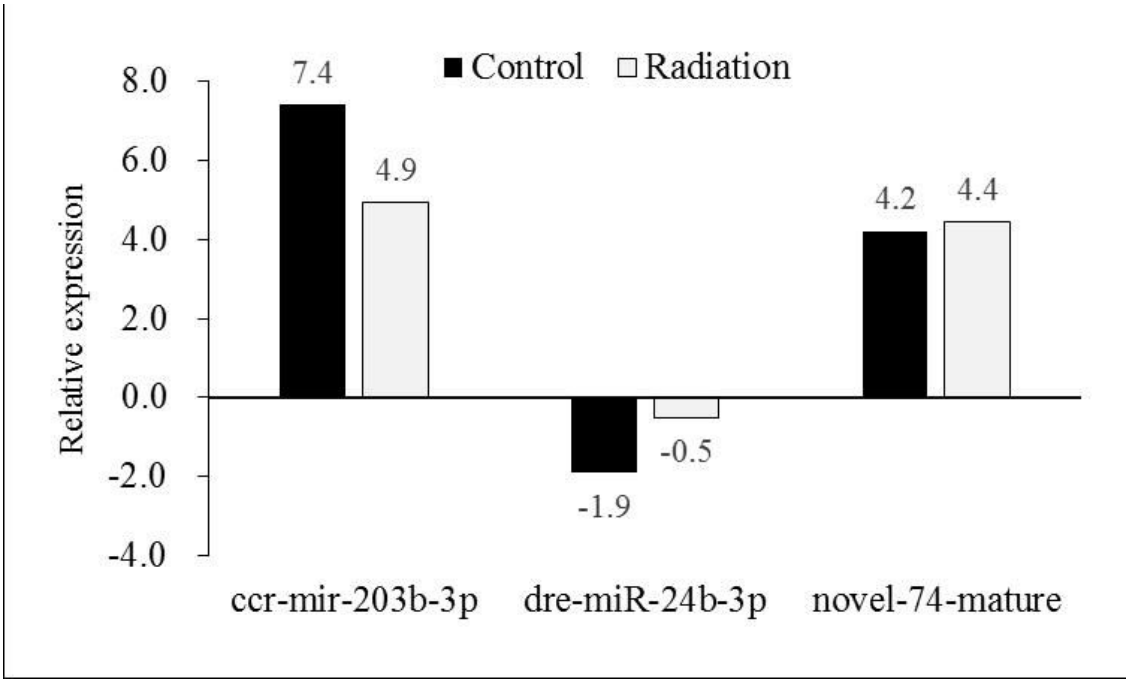

Figure 4

Expression verification by q-PCR

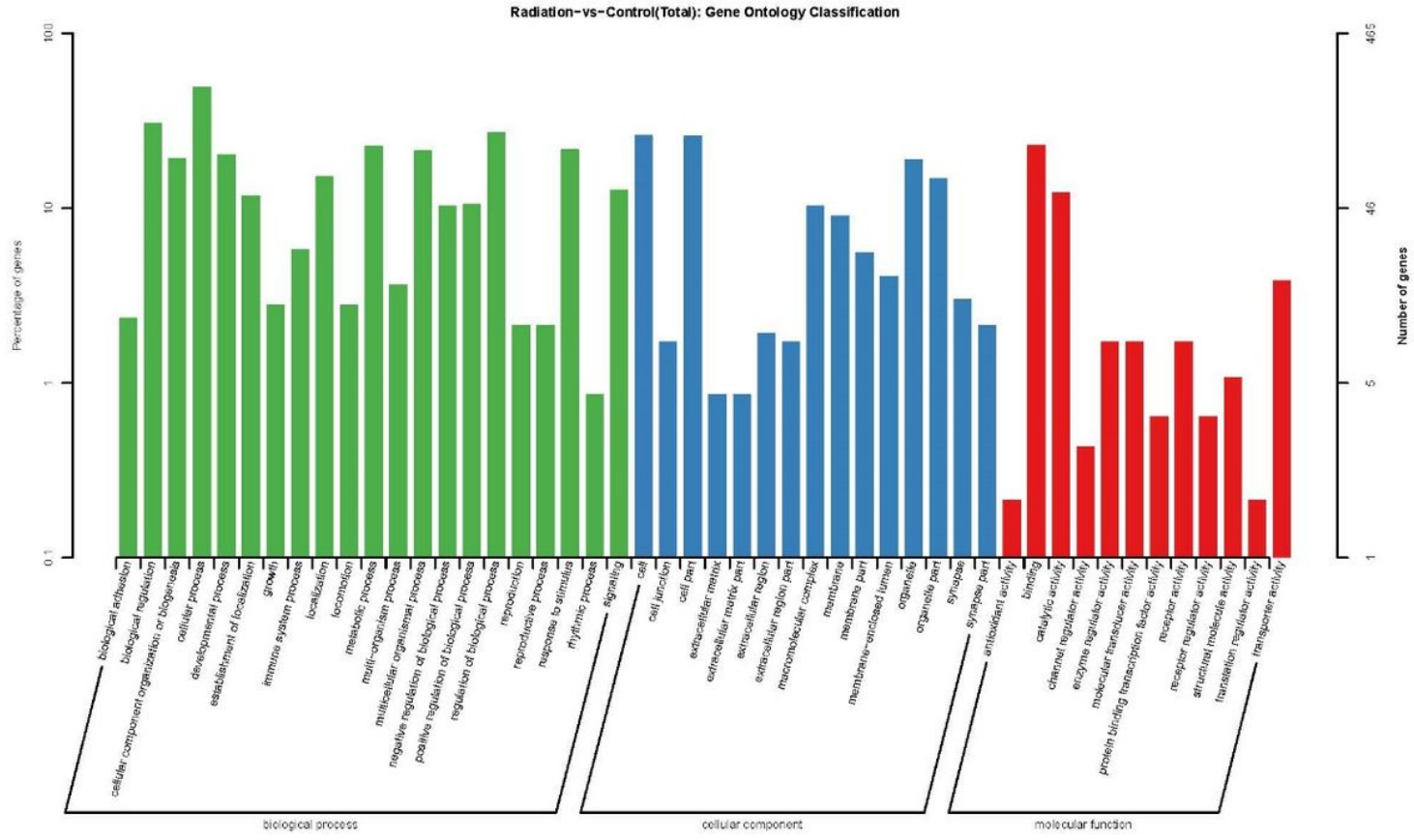

Figure 5

GO analysis of the differentially expressed miRNAs

Figure 6

GO enrichment analysis of the top 30 target genes

Figure 7

Prediction of KEGG pathways containing target genes of the differentially expressed miRNAs

Figure 8

KEGG enrichment analysis of the top 20 of target genes