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

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Abstract
Radiation can cause the differential expression of biological miRNA molecules. This research was based on the development of the 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; a total of 632 target genes were predicted in the prediction analysis. 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 the LRCC is sensitive to radiation, or can be used as a candidate experimental animal to study the biological effects of radiation, and the screened miRNA can be used as a pre-selected biomarker for radiation damage detection and radiation biological environmental monitoring.

Clinical Trials Registration
None.

Keywords Laboratory red crucian carp · Irradiation · MicroRNA

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 (Kamiya et al., 2015), exposure to below 0.2 Gy of nuclear radiation is relatively 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. With the development of nuclear science and radiation protection technology, the possibility of ultrahigh-dose nuclear radiation injury accidents is relatively low, such as at Fukushima and Chernobyl, but low-dose nuclear or ionizing radiation events are still possible. One-quarter Gy is a relatively safe and 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 in the liver of LRCC (Peng et al., 2016). 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 within 24 h after irradiation (Lemos et al., 2017a). On the basis of the previous research of our group and reference to relevant literature (Lemos et al., 2017b), 0.3 Gy was used as the low radiation induction dose and samples were collected 24 h after radiation induction for subsequent molecular experiments in this study.

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 a 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 (Frorczyk et al., 2016). Therefore, miRNAs could also be used as biomarkers for detecting biological radiation-induced damage.

Laboratory red crucian carp (LRCC) was domesticated from wild red crucian carp. 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). 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 and to screen out miRNA biomarkers related to the biological effects of low-dose nuclear radiation. RNA-seq and bioinformatics analysis techniques were combined to identify differentially expressed miRNAs that were sensitive to nuclear radiation and to predict their target genes and analyse their functions. Ultimately, it is expected that these screened LRCC’s radiation-sensitive miRNA biomarkers can be used to establish experimental animal models for the study of biological effects of low-dose radiation and biological radiation monitoring in the environment.

### Materials and methods

#### Experimental fish and treatments

Six tails one-year-old C1HD, with body lengths and weights of 8.71–10.01 cm and 26.94–37.76 g, respectively, 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.
The radiation group was given one-time whole-body irradiation with 0.3 Gy cesium-137 in a biological irradiator (China Nuclear Power Institute Equipment Manufacturing Plant, HXFS-IA, China). The radiation treatment was carried out in a radiation chamber, and the experimental fish were placed in a beaker containing 1000 ml of water with an oxygen concentration of 6 mg/L. The radiation dose rate of the radiometer is set at 0.5 cGy/s, and the irradiation time is 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. Firstly, 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 h, 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 sequences, 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,
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                    | 16,144,231  | 15,682,811   | 97.14       |
|        | tRNA           | 210   | 0                       |             |              |             |
|        | snRNA          | 1404  | 0.01                    |             |              |             |
|        | cis-reg        | 699   | 0                       |             |              |             |
|        | other RNA      | 2049  | 0.01                    |             |              |             |
|        | gene           | 10,503| 0.07                    |             |              |             |
|        | repeat         | 265,885| 1.65                   |             |              |             |
|        | known miRNA    | 10,325,275| 63.96               |             |              |             |
|        | unannotated    | 5,536,673| 34.3                  |             |              |             |
| C2     | rRNA           | 1031  | 0.01                    | 15,739,048  | 15,403,156   | 97.87       |
|        | tRNA           | 87    | 0                       |             |              |             |
|        | snRNA          | 782   | 0                       |             |              |             |
|        | cis-reg        | 370   | 0                       |             |              |             |
|        | other RNA      | 557   | 0                       |             |              |             |
|        | gene           | 4642  | 0.03                    |             |              |             |
|        | repeat         | 176,246| 1.12                   |             |              |             |
|        | known miRNA    | 11,000,231| 69.89              |             |              |             |
|        | unannotated    | 4,555,102| 28.94                 |             |              |             |
| C3     | rRNA           | 1016  | 0.01                    | 14,167,690  | 13,682,853   | 96.58       |
|        | tRNA           | 165   | 0                       |             |              |             |
|        | snRNA          | 907   | 0.01                    |             |              |             |
|        | cis-reg        | 512   | 0                       |             |              |             |
|        | other RNA      | 615   | 0                       |             |              |             |
|        | gene           | 16,765| 0.12                   |             |              |             |
|        | repeat         | 341,553| 2.41                   |             |              |             |
|        | known miRNA    | 8,793,238| 62.07                 |             |              |             |
|        | unannotated    | 5,012,919| 35.38                 |             |              |             |
| R1     | rRNA           | 1157  | 0.01                    | 14,716,192  | 14,317,228   | 97.29       |
|        | tRNA           | 281   | 0                       |             |              |             |
|        | snRNA          | 1691  | 0.01                    |             |              |             |
|        | cis-reg        | 619   | 0                       |             |              |             |
|        | other RNA      | 1138  | 0.01                    |             |              |             |
|        | gene           | 9951  | 0.07                    |             |              |             |
|        | repeat         | 324,946| 2.21                   |             |              |             |
|        | known miRNA    | 9,809,742| 66.66                 |             |              |             |
|        | unannotated    | 4,566,667| 31.03                 |             |              |             |
| R2     | rRNA           | 947   | 0.01                    | 15,251,035  | 14,845,007   | 97.34       |
|        | tRNA           | 162   | 0                       |             |              |             |
|        | snRNA          | 1368  | 0.01                    |             |              |             |
|        | cis-reg        | 564   | 0                       |             |              |             |
|        | other RNA      | 1126  | 0.01                    |             |              |             |
|        | gene           | 4281  | 0.03                    |             |              |             |
|        | repeat         | 174,387| 1.14                   |             |              |             |
|        | known miRNA    | 10,567,717| 69.29                |             |              |             |
|        | unannotated    | 4,500,483| 29.51                 |             |              |             |
| R3     | rRNA           | 970   | 0.01                    | 14,477,040  | 14,153,426   | 97.76       |
|        | tRNA           | 122   | 0                       |             |              |             |
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. The miRNAs with significant differential expression were screened according to their expression levels and differences. The screening conditions are as follows: (1) Padj <0.1 or p-value < 0.01, p-value < 0.05 when the number of genes is small; (2) In the two groups of samples, at least one group of average expression (TPM) > 2; (3) FC (abs) >2, FC (abs)>1.5 when the number of genes is small.

Q-PCR verification of differentially expressed miRNA

Three miRNAs were selected from the total differentially expressed, 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.

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). These 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

| Table 2 (continued) |
|---------------------|
| Sample | Annotation type | Reads | Percentage of total (%) | Total reads | Aligned reads | Aligned (%) |
|---------|----------------|-------|--------------------------|-------------|---------------|-------------|
| snRNA   | 1147           | 0.01  |                          |             |               |             |
| cis-reg | 605            | 0     |                          |             |               |             |
| other RNA | 698      | 0     |                          |             |               |             |
| gene    | 5335           | 0.04  |                          |             |               |             |
| repeat  | 162,716        | 1.12  |                          |             |               |             |
| known miRNA | 10,208,710 | 70.52 |                          |             |               |             |
| unannotated | 4,096,737 | 28.3  |                          |             |               |             |

| Table 3 Total number 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          |
995 newly predicted miRNAs were identified from the 6 samples (Table 3).

**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, abu-miR-19b, ssa-miR-125b-2-3p, dre-miR-18c, dre-miR-126a-3p, gmo-miR-27d-3p, 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*), etc. (Table 4).

| miRNA_id | log2 Fold Change | p-value | Regulation | Sequence |
|----------|------------------|---------|------------|----------|
| abu-miR-152 | Inf | 0.019 | Up | TCAGTGCATAACAGAAGTTT |
| abu-miR-454b | 3.32 | 0.048 | Up | TAGTGCAATATTGCTTATAGGG |
| abu-miR-99b | 4.25 | 0.006 | Up | AACCGTGATCGCCGCTTCTT |
| dre-miR-126a-3p | 3.77 | 0.000 | Up | TCGTACCGTGAGTAATAGCT |
| dre-miR-18c | 2.16 | 0.034 | Up | TAAGTGCAATCTTGTTAGTTA |
| dre-miR-202-5p | 5.49 | 0.001 | Up | TTTCTATGCTATACCCCTT |
| dre-miR-24b-3p | 5.71 | 0.002 | Up | TGGCTCAATTGAGCAAGAACCG |
| dre-miR-9-7-3p | 3.06 | 0.014 | Up | TAAAGCTAGAGAACGGAAAGTT |
| gmo-miR-29d-3p | 2.59 | 0.030 | Up | TAGCACCATTGAAACTCGT |
| ipu-miR-181a | 1.92 | 0.039 | Up | AACATTCAGCAGCTTGCTT |
| ipu-miR-29a | Inf | 0.019 | Up | ACTGATTTCCTGGTT |
| novel-402-mature | 2.95 | 0.041 | Up | AACACTGCTGACATTGATCTT |
| novel-74-mature | 10.45 | 0.045 | Up | AACACGGGAGCTGACTGCTCA |
| ola-miR-200b | 3.02 | 0.046 | Up | TAATACGTGCTGTTAATAGTG |
| ssa-miR-125b-2-3p | 2.79 | 0.047 | Up | CAGTGAGTCTCTTGGAAC |
| ssa-miR-29a-3p | 2.80 | 0.049 | Up | TAGCACCATTGAAACTCGT |
| abu-miR-19b | −3.33 | 0.005 | Down | TGGCAAAATCATGGACACTG |
| abu-miR-205 | −2.88 | 0.021 | Down | TCTTCTATGTCACGGAGCTC |
| ccr-miR-203b-3p | −2.60 | 0.036 | Down | GTGAATGTGAACACTT |
| ccr-miR-222 | −4.56 | 0.001 | Down | AGCTACATCTGGCTACCTG |
| dre-miR-27c-3p | −4.08 | 0.027 | Down | TTCACAGTGTTAATGTTCTG |
| gmo-miR-140-5p | −7.68 | 0.000 | Down | CAGTGGTGTACCTTATG |
| gmo-miR-18a-2-5p | −2.96 | 0.032 | Down | TAAGTGCAATCTGAGCTAGAT |
| gmo-miR-21-3p | Inf | 0.002 | Down | CGACAACAGTCTGAGCTTG |
| gmo-miR-27d-3p | −4.22 | 0.001 | Down | TTCACAGTGCTAAATGTGG |
| novel-261-mature | −3.79 | 0.016 | Down | TTCACCAGCTGAAATATCTGAT |
| novel-370-mature | −3.61 | 0.039 | Down | AAAGTGCTGCTCTTCTT |
| novel-451-mature | −Inf | 0.046 | Down | TTTGCTAGGAAATGCTT |
| novel-565-mature | −Inf | 0.036 | Down | TGACGCTGCTAGCAGCTT |
| novel-88-star | −Inf | 0.002 | Down | TAAAGACGGTTATCTAATCAG |
| ola-miR-126-3p | −4.20 | 0.010 | Down | TCGTACGAGTGAATATAG |
| ola-miR-140-3p | −4.27 | 0.014 | Down | ACCACAGGTTAGCAGCCG |
| ola-miR-148 | −4.62 | 0.009 | Down | TCAGTGACATTACAGACCTT |
| ola-miR-27d-3p | −6.18 | 0.002 | Down | TTCACAGTGCTAAATGTC |
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).

**Prediction and functional analysis of target genes of differentially expressed miRNAs**

Mirnada 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 (Figs. 5 and 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 (Figs. 7 and 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**

This study showed that exposure to 0.3 Gy irradiation stress did not cause visible surface damage or death in the LRCC, which was consistent with the previous research. 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 were 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-22, gmo-miR-

Fig. 5 GO analysis of the differentially expressed miRNAs

Fig. 6 GO enrichment analysis of the top 30 target genes
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.,...
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). Wenjun 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. On this basis, it is necessary to verify the functions of key target genes regulated by differentially expressed miRNAs to determine the application feasibility of these screened miRNAs.

The effects of radiation observed in previous research show that LRCC also has high sensitivity to nuclear radiation. For example, 1.94 Gy can cause changes in blood physiology and biochemistry, enzymology, protein expression, and other parameters (Peng et al., 2016; Tao et al., 2021; Liao et al., 2020; Wu, 2016). A radiation dose of 0.3 Gy can cause nuclear constriction in hepatocytes and changes in liver miRNA expression; 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.

Data availability

All the datasets used and/or analyzed throughout the present study are available from the corresponding author on reasonable request.

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Author contributions

YDW, KJC and DSW designed the study, carried out the analyses, and prepared and draft the manuscript. YDW, XLL, KJC, DSW, and ZHZ performed the technical discussions. YDW, XLL, and KJC participated in data simulation and discussions. YDW, KJC, YXL, and JPY were involved in the statistical analysis. All the authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest

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.

Animal Research (Ethics)

The procedures were conducted in accordance with the approved guidelines. Individual experimental fish was housed in an aquarium (0.3 m$^2$) 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.

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