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Proteomics-based molecular and functional characteristic profiling of muscle tissue in Triploid crucian carp†

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Triploid crucian carp (TCC) is a kind of artificially bred fish with huge economic value to China. It has several excellent characteristics, such as fast growth, strong disease resistance and delicious taste. However, as a regionally specific fish, the underlying molecular mechanisms of these characteristics are largely unknown. In this study, we performed quantitative proteomics on the muscle tissues of TCC and its parents, allotetraploid (♂), red crucian carp (♀) and common carp. Combined with multiple bioinformatic analysis, we found that the taste of TCC can be mainly attributed to umami amino acid-enriched proteins such as *PURBA*, *PVALBI* and *ATP5F1B*, and that its rapid growth can be mainly ascribed to the high expression and regulation of metabolism-related proteins such as *NDUFS1*, *ENO1A* and *CS*. These play significant roles in substrate and energy metabolism, as well as in bias transformation. Subsequently, we identified several proteins, including *MDH1AA*, *GOT1* and *DLAT*, that may serve as potential regulators of innate immunity by regulating the biosynthesis and transformation of significant antibiotics and antimicrobial peptides. In conclusion, this study can serve as a significant reference for similar investigations and shed light on the molecular and biological functions of individual proteins in TCC muscle tissue.

1. Introduction

Triploid crucian carp (TCC) is a kind of artificially produced freshwater fish with great economic benefits. It was firstly cultured at the State Laboratory of Developmental Biology of Freshwater Fish in Hunan, China.[1] TCC is obtained *via* distant hybridization between allotetraploids (4nAT, ♂) and red crucian carps (RCC, ♀).[2] The 4nAT itself was bred *via* distant hybridization between RCCs and common carp (CC).[1] Due to its characteristics of great taste, fast growth and strong disease resistance, TCC is a favorite among Chinese fishermen and consumers.[1] Compared to most natural species, TCC is non-reproductive, and most of its energy is used for growth rather than reproduction.[3–5] TCC has several other advantages, such as a short growth cycle, umami meat, strong antiviral ability and low-temperature resistance.[6,7] Therefore, further molecular study of TCC is urgent and necessary, and will provide significant guidance for genetic breeding and variety improvement.[2]

Muscles are important tissues that perform vital activities during exercise and energy metabolism.[8] The muscles of teleostean account for 15 to 20 percent of its body weight, and most of them are edible.[9] The protein and amino acid content of fish muscles are very important for the quality of fish meat. The umami flavor of fish is generally considered to be the primary and most important aspect of its flavor quality.[10] Of the five basic flavors—salty, sour, sweet, bitter and umami—umami is the most crucial factor, and it is often taken as a sign of protein-rich and L-amino acids in food.[11,12] "Umami" is recognized as a basic taste typified by glutamic acid, and monosodium glutamate, a salt. Fish meat is rich in creatine and amino acids, which is the main reason why the taste of fish is appealing.[10,13] The content of umami amino acids (Ala, Glu, Lys, Asp, Leu) can affect the taste of anchovies and Pufferfish.[14,15] In that respect, our study indicates that the muscle proteome of TCC is directly related to both the delicious taste and growth of fish. It is well known that body growth is related to the rate of muscle growth; the skeletal muscle is the largest cell population in the body.[16–18] Muscles are also involved in the immune capacity of fish, in addition to head-, kidney-, spleen- and

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intestinal-associated lymphoid tissues.[19,20] The immune response of skeletal fish muscles is stimulated differently by exercise or pathogenic infection.[21]

Proteins are the main and direct performers of most physiological functions. Proteomics has become a powerful method in the large-scale investigation of proteins with respect to biological and biotechnological research.[22] High-throughput proteomic methods are used to quantify and identify proteins and mine protein–protein interactions (PPI) in different cells, tissues and organisms under particular physiological and pathological conditions. Complex biochemical pathways are caused by changes in multiple proteins and biological pathways.[23] The sequential window acquisition of all theoretical mass spectra (SWATH-MS) method is well suited for projects requiring large numbers of samples; it supports the quantitative analysis of wide peptide coverage and sensitivity, and it accurately and reproducibly quantifies the proteome or peptide groups expressed in each sample.[24,25]

Most of the previous studies on TCC have focused on the function analysis of individual genes or proteins or on the genome level.[26,27] Proteins are the direct agents and executors of physiological and biochemical functions, and protein amount levels and protein–protein interactions are dynamic under various physiological and pathological situations; thus, it is significant to analyze real-time protein amount levels and evaluate PPIs under specific situations on a systemic level. In this study, we used SWATH-MS methods for protein identification and quantification.[28] Subsequently, the biological functions and molecular mechanisms of the measured proteins were comprehensively analyzed from the perspectives of computer science, statistics and biological knowledge.

2. Materials and methods

2.1. Experimental animals and sample preparation

In this study, we performed high-throughput proteomic profiling using fish muscles from TCC and its parents (RCC, 4nAT and CC). TCCs were cultured at the State Laboratory of Developmental Biology of Freshwater Fish in Hunan, China. TCC, CC, RCC and 4nAT, aged 10–11 months, were collected for experiments. The license number was 2020318, and animal experimenters were certified under a professional training course for laboratory animal practitioners held by the Institute of Experimental Animals, Hunan Province, China. All fish were euthanized using 2-phenoxyethanol before dissection. The muscle tissue samples were taken and collected from the above four species by professional technicians. The fresh tissue samples were immediately frozen with liquid nitrogen and then stored at −80 °C. In this study, three fish per species and three muscle tissue samples from each fish were used for proteomic analysis.

About 200 mg of frozen tissue samples was ground into cell powder and then transferred to a 1.5 mL centrifuge tube; 300 μL of RIPA lysis buffer (Radio-Immunoprecipitation Assay, Biyotime, China) was added, and an appropriate protease inhibitor (PMSF) (Amresco) was set to 1 mM. An ultrasonic crusher with a power of 80 W was used to crush the sample at 4 °C. The ultrasonic crusher was first turned on for 1.0 s and then turned on again at 1.0 s intervals. The opening and closing were carried out alternately for 3 minutes. After repeated shock, the samples were fully dissolved. After that, the samples were centrifuged at 12 000 g at room temperature for 10 min, and the precipitation was discarded in order to collect the supernatant. Then, the supernatant was centrifuged again to collect its own supernatant. The total protein content in the supernatant was measured with a bicinchoninic acid (BCA) quantitative kit (ThermoScientific). The total protein extract, 50 μg, was mixed with 30 μL of reduction buffer (10 mM DTT (dithiothreitol), 8 M urea, 50 mM NH_4HCO_3, pH 8.5) and filtered in a 10 KD ultrafiltration tube. The solution was incubated for 1 hour at 55 °C, then IAA (iodoacetamide) was added to the solution at a final concentration of 50 mM. Subsequently, all samples were incubated for 20 minutes at room temperature in the dark. The solution was centrifuged on the filter at 12 000 rpm for 20 min at 4 °C and then the flow-through was discarded from the collection tube. We then added 200 μL of 8 M urea to a 50 mM NH_4HCO_3 buffer, which was centrifuged at 12 000 rpm for 20 min. After the washing step, the filter units were transferred to an additional collection tube containing 200 μL of 50 mm NH_4HCO_3, and the operation was repeated. An amount equal to 1 μg of trypsin was added to each tube.[29] The solution was incubated at 37 °C for 14 hours. After digestion, peptides were reconstituted and desalted with 1 ml of 0.1% TFA and an RP-C18 solid phase extraction column, respectively.

2.2. Protein identification and quantification with SWATH mass spectrometry

Determination was performed with an Agilent ZORBAX Extended C18 column (5 μm C18, 2.1 × 150 mm) using buffer A (10 mmol L^{-1} NH_4OH, 95% H_2O, pH = 10.0) at a flow rate of 0.3 mL min^{-1}. The peptide was first eluted into 32 fractions using the column in buffer B (10 mmol L^{-1} NH_4OH in 90% ACN, pH = 10.0) with a slope gradient of 0–35% over 70 minutes and then finally incorporated into 12 pools. The initial 1.5 μg separation of peptide material was dissolved in a solvent (98% water, 2% acetonitrile, 0.1% formic acid) and directly loaded into a trap column (ChromXP C18, 3 μm, 120 Å, SCIEX, USA) at a flow rate of 4 μL min^{-1} for 5 minutes. The samples were separated and analyzed in a column (75 μm x 15 cm, using C18, 3 μm 120 Å, ChromXP, Eksigent) using the Ekspert Nanolc 415 system (SCIEX, Harmony). Gradient B of the acetonitrile solvent (98%, 2% water, 0.1% formic acid) increased from 5% to 38% in 100 minutes. After that, the generated peptide was introduced into the TripleTOF 6600 Mass Spectrometer (Sciex, Concord, Ontario, Canada) and fitted with a Nanospray III ion source in active DIA (data-independent acquisition) mode based on the following settings: an ion spray voltage of 2.3 kV, curtain gas at 35 PSI, sprayer gas at 12 PSI, and an interface heating temperature of 150 °C. Information-dependence analysis was used for mass spectrum scanning. The survey scan was completed within 250 ms, and when the 260 cps threshold was

exceeded and the charge state was 2–4, a maximum of 40 precursor ion scans (50 ms) were collected. In addition, rolling collision energy and kinetic repulsion parameters were applied to the collision-induced dissociation and 18 s of all precursor ions, respectively. As per the manufacturer's instructions, IRT calibration peptide (Biognosys, Schlieren, Switzerland) was added to each sample for SWATH quantification. In a scanning range of 350–1350 *m*/*z*, 60 separate windows were set according to *m*/*z* density, including 1 Da window overlapping between two adjacent mass windows. All mass spectral data (*e.g.*, the generated WIFF file from TripleTOF 6600) were processed by the ProteinPilot software v.5.0 (Sciex Inc., USA) to construct a spectral library. A combined database, including homemade transcriptomic data on allotetraploids and a UniprotKB protein database regarding Danio rerio (txid7955), was used to search through about 62 000 entries in the database with the following search settings: sample type: identification; cys alkylation: iodoacetamide; digestion: trypsin; fixed modifications: Acetylation (protein N-terminus); variable modification: Carbamoylmethylation(Cys); mass tolerance for peptide and fragment ions: 10 ppm and 0.02 Da, respectively; tryptic restriction with two maximal missed cleavages. The entire process was performed with ID TripleTOF 6600. The overall false discovery rate (FDR) for the peptide and protein levels estimated by the combined PSPP tool was 1.0%. Finally, all SWATH-MS data were analyzed using Spectronaut Pulsar X software (Biognosys, Switzerland) to identify and quantify peptides and proteins compared to those in the spectral library. The default settings were used, including the exclusion of repeat analysis, *m*/*z* and retention-time calibration with HRM peptides inserted.

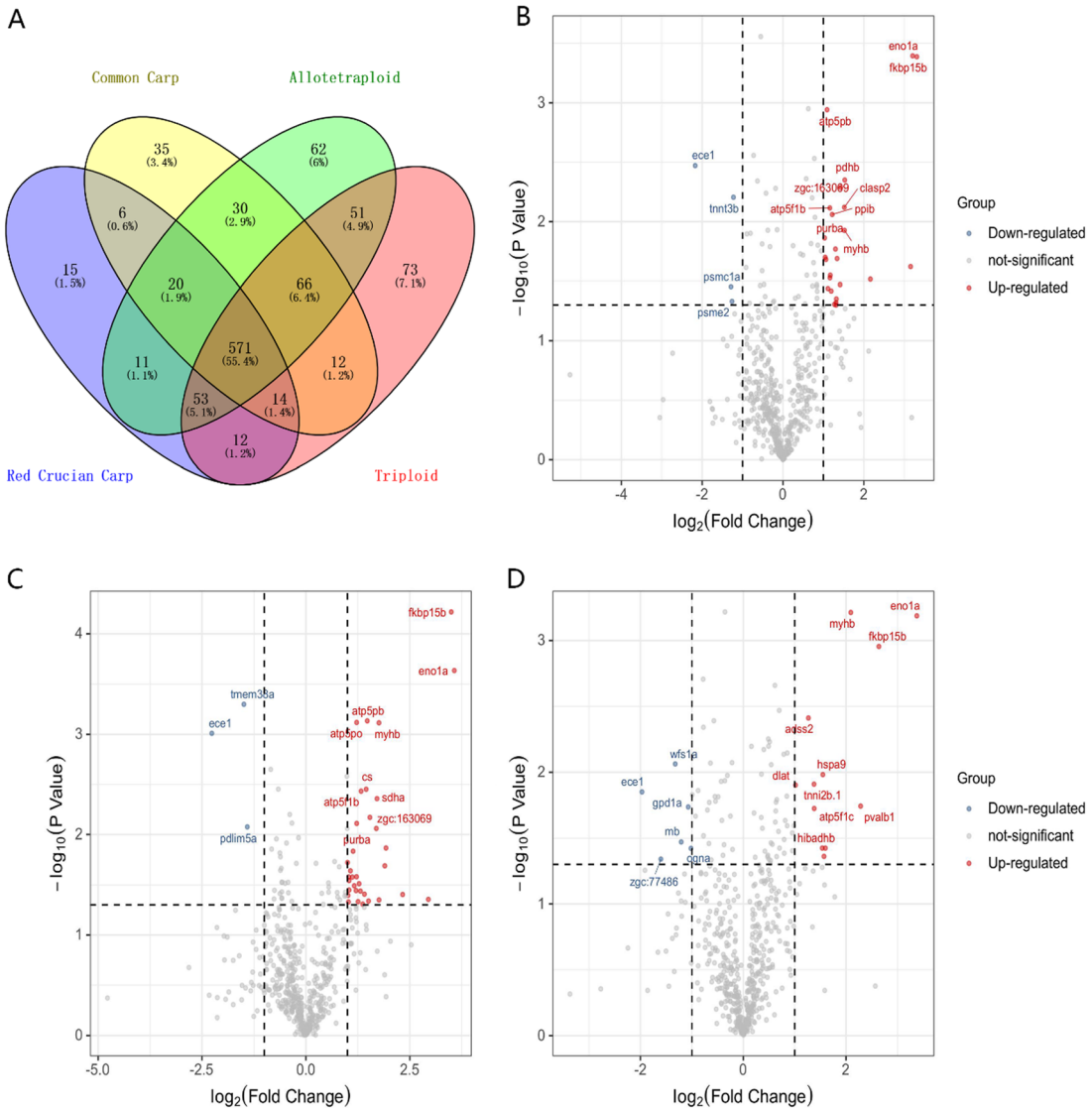

Fig. 1 Statistical summary of muscle proteins and DEPs with respect to TCC and its direct/indirect parents. (A) Venn diagram expressing the species-specific proteins of four fish (RCC, CC, 4nAT, TCC). (B–D) The DEPs between TCC and RCC, TCC and CC and TCC and 4nAT, respectively. The first 10 downregulated proteins and the first 10 upregulated proteins are marked. Red dots represent upregulated proteins and blue dots represent downregulated proteins.

Table 1 Statistical summary of proteome profiling in four fishes

Species	Identified proteins	Quantified proteins	Species-specific proteins
Red crucian carp	976	702	15
Common carp	1037	754	35
Allotetraploid	1127	864	62
Triploid crucian carp	1054	852	73

Table 2 Differentially regulated proteins of triploid crucian carp (TCC) compared to red crucian carp (RCC), common carp (CC) and allotetraploid (4nAT)

Comparable groups	Up-regulated proteins	Down-regulated proteins
TCC *vs.* RCC	25	4
TCC *vs.* CC	34	3
TCC *vs.* 4nAT	11	5

Interference correction was activated and a local normalization strategy for crossover operation normalization was adopted. All results had a *q*-value of 0.01 (equivalent to an FDR of 1% at the peptide level). Due to limitations with respect to the sensitivity, resolution and coverage of proteomic techniques, missing values are an inevitable problem in quantitative proteomic research. Therefore, to improve the accuracy and reliability of our proteomic data, we performed triple biologic and technical runs on each muscle sample. The mean value of the biologic triplicate runs was used to estimate each protein amount level.

2.3. Identification of differentially expressed proteins (DEPs) and species specifically expressed proteins

To determine the differentially expressed proteins (DEPs) between TCC and its parents, RCC, 4nAT and CC, Student's *t*-test and fold change (FC) calculation statistical methods were applied; the corresponding values ($p < 0.05$ and $|\ln_2 FC| > 1$) were set as a cutoff line to detect DEPs. A subsequent Venn diagram was produced to show the shared and unique proteins among the different species.

2.4. Amino acid composition and content analysis of important proteins

The ExPASY website[30] was used to calculate various physical and chemical parameters for each given protein, such as molecular weight, theoretical isoelectric point, amino acid composition and atomic composition. The upregulated proteins were queried with ProtParam[30] in order to evaluate the composition and content of the umami amino acids.

2.5. Gene enrichment analysis and functional annotation

As a complete biological knowledgebase analytical tool, DAVID (Database for Annotation, Visualization and Integrated Discovery) can systematically extract biological significance from large lists of genes/proteins. It provided multiple functional annotation tools and an integrated data-mining environment for the purposes of exploring biological significance within a large list of genes.[31,32] In order to maximize the coverage of biologically meaningful information, all DEPs were input into DAVID for gene functional annotation, enrichment and pathway analysis. The biologically significant terms were filtered with the following statistical thresholds: Benjamini-adjusted $p < 0.05$ and an enriched gene count > 2.

2.6. PPI analysis and network reconstruction

The differentially expressed proteins and species-specific proteins were mapped into the STRING database,[33] and the PPI interaction score threshold was set to 0.4. Finally, the results were exported into TSV format to facilitate visualization and reconstruction in Cytoscape (Cytoscape 3.7.2). The plug-in ClueGo (ClueGo 2.5.7) from the Cytoscape (Cytoscape 3.7.2) platform was used for subnetwork reconstruction and

Table 3 Amino acid content of the top twenty upregulated proteins in TCC

Protein	Protein full name	Ala (%)	Asp (%)	Glu (%)	Gly (%)	Sum (%)	Ratio (TCC *vs.* RCC/TCC *vs.* CC/TCC *vs.* 4nAT)	*P*-value (TCC *vs.* RCC/TCC *vs.* CC/TCC *vs.* 4nAT)
PURBA	Purine-rich element-binding protein Ba	5.7	7.9	8.2	18.3	40.1	2.04/2.33/NA	1.4E-02/7.8E-03/NA
PVALB1	Parvalbumin	22	0.9	2.8	7.3	33	2.49/NA/4.87	4.8E-02/NA/1.8E-02
ATP5F1B	ATP synthase subunit beta	11	4.8	7	9.9	32.7	2.23/2.51/NA	7.7E-03/3.7E-03/NA
ATP5FA1	ATP synthase subunit alpha	10.5	6.5	6.4	9.1	32.5	2.29/2.58/NA	3.8E-02/4.9E-02/NA
DLDH	Dihydrolipoyl dehydrogenase	10.7	3.2	6.1	11.6	31.6	2.09/2.24/NA	2.1E-02/3.2E-02/NA
PDHB	Pyruvate dehydrogenase E1 component subunit beta	11.1	5.6	6.7	7.5	30.9	2.89/3.73/NA	4.5E-03/2.0E-02/NA
MYH7BB	Myosin, heavy chain 7B, cardiac muscle, beta b	11.3	3.5	4.4	11.1	30.3	8.93/7.72/2.97	2.4E-02/4.4E-02/4.4E-02
ATP5PB	ATP synthase subunit b	7.1	6.3	12.9	3.7	30	2.13/2.78/NA	1.1E-03/7.3E-04/NA
FKBP15B	Peptidylprolyl isomerase	8.1	5.3	12.2	4.3	29.9	9.94/11.31/6.23	4.1E-04/6.1E-05/1.1E-03
ADSS2	AMPSase 2	6.2	4.6	7	11	28.8	NA/2.1/2.41	NA/2.3E-02/3.9E-03
ATP5F1C	ATP synthase subunit gamma	10.6	5.5	5.8	5.8	27.7	2.49/2.66/2.6	5.0E-02/3.9E-02/1.9E-02
ENO1A	2-Phospho-D-glycerate hydro-lyase	9.5	3.5	6.2	8.3	27.5	9.25/11.9/10.39	4.0E-04/2.3E-04/6.5E-04
NNT2	Proton-translocating NAD(P)(+) transhydrogenase	9.4	2.7	4.3	10.9	27.3	2.24/2.48/NA	3.0E-02/3.6E-02/NA
HIBADHB	3-Hydroxyisobutyrate dehydrogenase	9.4	2.7	4.3	10.9	27.3	4.48/NA/2.9	3.0E-02/NA/3.8E-02
CS	Citrate synthase	8.3	4.8	7.5	6	26.6	2.46/2.73/NA	1.7E-02/3.5E-03/NA
MYHB	Myosin, heavy chain b	4.8	2.9	5.3	12.5	25.5	2.87/3.38/4.26	1.2E-02/7.7E-04/6.1E-04
ATP5PO	ATP synthase subunit O, mitochondrial	9.1	3.8	4.3	6.2	23.4	2.06/2.33/NA	2.0E-02/7.6E-04/NA
ZGC:163069	ATP synthase subunit beta	7.3	1.7	8.5	5.6	23.1	2.7/2.91/NA	5.2E-03/6.8E-03/NA
GAMT	Guanidinoacetate *N*-methyltransferase	7.3	1.7	8.5	5.6	23.1	2.25/2.2/NA	2.8E-02/1.5E-02/NA
NDUFA4L	NADH: ubiquinone oxidoreductase subunit A4	4.2	5.2	1	6.2	16.6	2.66/2.43/NA	3.4E-02/3.1E-02/NA

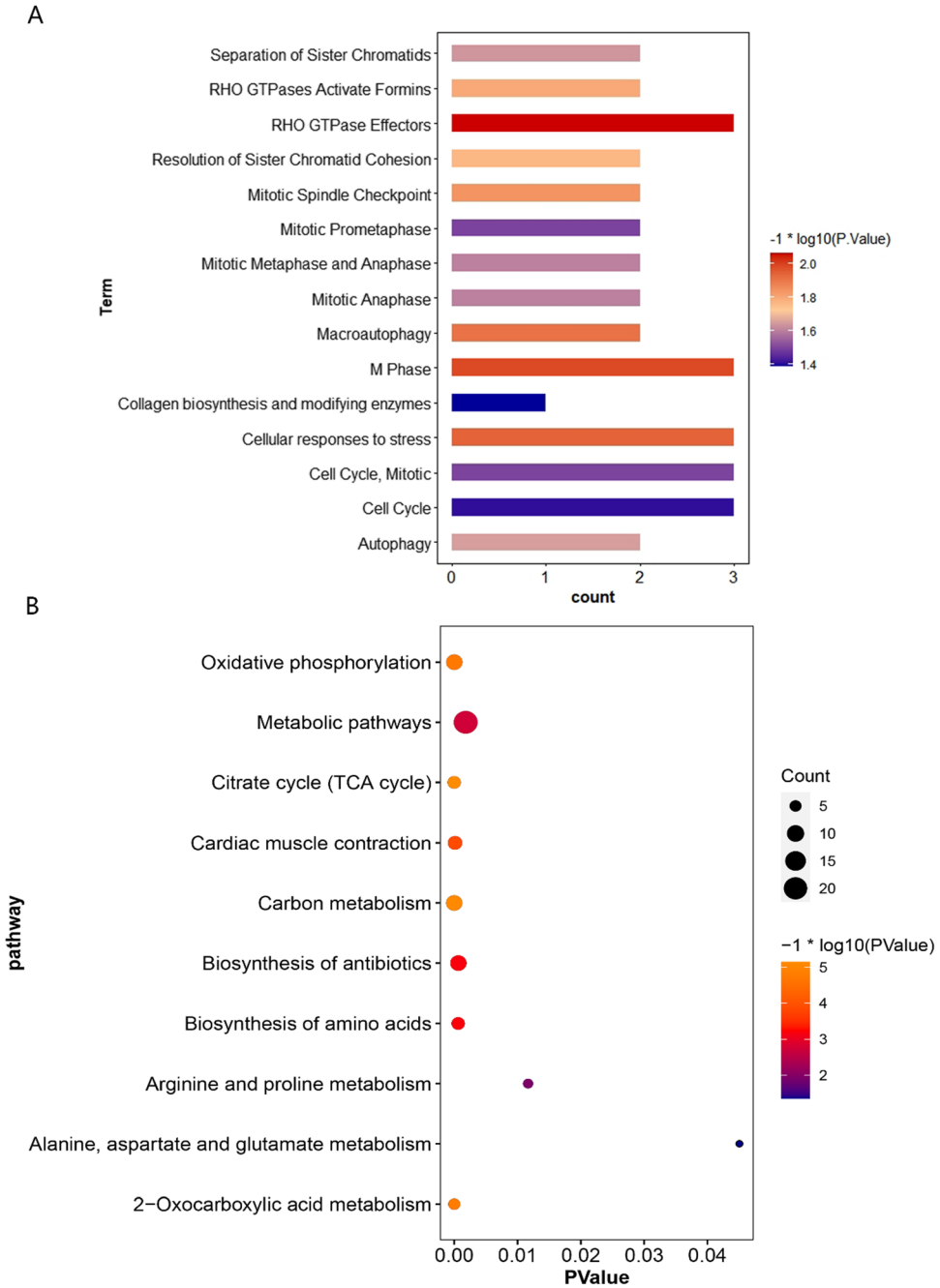

Fig. 2 Priority of crucial terms and pathways related to growth heterosis based on species-specific and differentially expressed proteins. (A) Biological processes related to cell division based on species-specific and differentially expressed proteins. (B) Significant pathways enriched in regulated, species-specific and differentially expressed proteins. These important proteins are involved in cell division and proliferation in order to promote TCC growth and are rich in material and energy, with metabolic pathways that indicate energy biased transfer.

visualization.[34,35] PPI networks of protein–protein and protein–pathway interactions were constructed and visualized based on the following key parameters: background database: Danio rerio; statistical thresholds: 0.05; network specificity: medium. Network reconstruction and visualization allowed us to better understand the complex biological functions and synergistic regulation of species-specific proteins and DEPs.

3. Results

3.1. Statistical summary of proteomics profiling

Based on the SWATH-MS method, 976, 1037, 1127 and 1054 proteins were identified in RCC, CC, 4nAT and TCC, respectively. To reduce false protein detection, 702, 754, 864 and 852 proteins identified and quantified in two or more runs were used for further analysis.

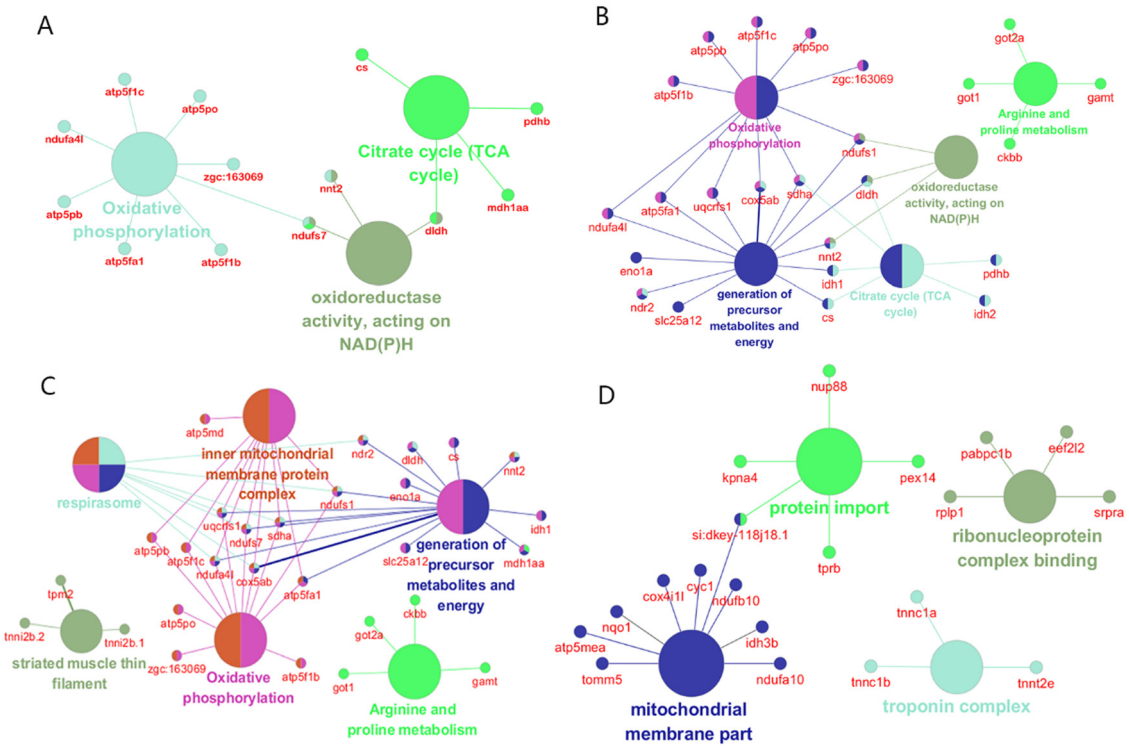

Fig. 3 Network reconstruction and visualization based on DEPs and species-specific expressed proteins. A and B represent networks based on the DEPs between TCC and RCC and TCC and CC; C and D represent networks constructed by DEPs (TCC and CC, TCC and CC, TCC and 4nAT) and species-specific proteins (TCC), respectively.

Table 4 Two significant pathways and the corresponding DEPs involved in the innate immune system

Pathway	Gene
Biosynthesis of antibiotics	*ENO1A*, *MDH1AA*, *GOT1*, *GOT2A*, *IDH1*, *IDH2*, *IDH3B*, *DLAT*, *SDHA*
Biosynthetic amino acids	*ENO1A*, *GOT1*, *GOT2A*, *IDH1*, *IDH2*, *IDH3B*

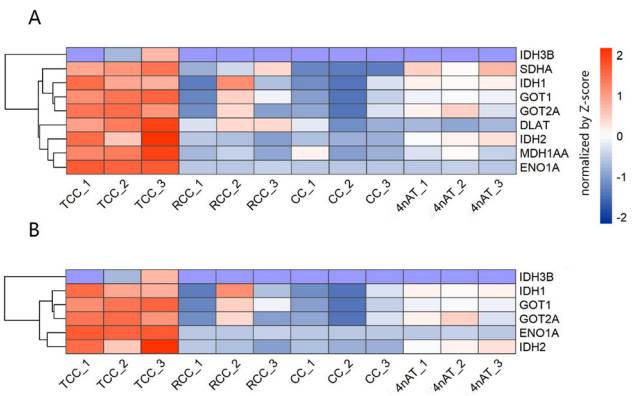

Fig. 4 Changes in the expression of important immune-related proteins in different fish. A and B represent the expression level of those proteins involved in biosynthetic antibiotic and biosynthetic amino acid pathways respectively.

A horizontal comparison of the identified proteins was performed for the above four fishes. As shown in Fig. 1A, there were 15 species-specific proteins in RCC, 35 species-specific proteins in CC, 62 species-specific proteins in 4nAT and 73 species-specific proteins in TCC (Table 1).

3.2. Identification of differentially expressed proteins

To further determine the differential regulation of proteins between TCC and its parents, a comparative proteomic analysis was conducted in order to detect DEPs based on the Student's *t*-test ($p < 0.05$) and the fold change calculation (ratio > 2 or < 0.5) (Fig. 1B–D). Compared to RCC, there were 25 upregulated proteins and 4 downregulated proteins in TCC. Compared to CC, there were 34 upregulated proteins and 3 downregulated proteins in TCC. Compared to 4nAT, there were 11 upregulated proteins and 5 down-regulated proteins in TCC (Table 2).

3.3. Proteins highly expressed in TCC are rich in umami amino acids

To better explain why TCC's flesh is more tender and delicious than its parents on a molecular level, we analyzed the amino acid composition of all the upregulated proteins in TCC. In Table 3, the amino acid types and proportions for the top twenty upregulated proteins are listed. The results indicate that

the umami amino acids, such as Ala, Asp, Glu and Gly, take a large proportion in most upregulated proteins. For instance, the highly abundant *PURBA* protein contained 5.7% Ala, 7.9% Asp, 8.2% Glu and 8.3% Gly; *PVALBI* contained 22% Ala, 0.9% Asp, 2.8% Glu and 7.3% Gly; *ATP5F1B* contained 11% Ala, 4.8% Asp, 7% Glu and 9.9% Gly; *ATP5FAI* contained 10.5% Ala, 6.5% Asp, 6.4% Glu and 9.1% Gly. The Glu and Asp content in *PURBA* and *ATP5FAI* accounted for 16.1% and 12.9%, respectively. As we know, amino acids are divided into several groups based on their taste characteristics. Aspartic and glutamic acids represent glutamate-like components and are responsible for the most typical umami (or palatable) taste.[36] It has been reported that the content of L-Glu plays an important role in maintaining the umami taste of food. Therefore, the results indicate that most of the upregulated proteins in TCC (such as *PURBA*, *PVALBI*, *ATP5F1B*, *ATP5FAI* and *DLDH*) have a higher umami amino acid content. Our findings provide powerful evidence on a molecular level and help us to better uncover the reason why the triploid is tastier than its parents.

3.4. Species-specific proteins and DEPs play important roles in growth and metabolic pathways

We evaluated the roles of each DEP with respect to TCC growth heterosis. Gene functional annotation and enrichment analyses suggested that most DEPs are enriched in terms related to cell growth and substance metabolism. As expected, the upregulated proteins in TCC were mainly enriched in "separation of Sister chromatids" (P = 2.30E-02) and "M phase" (P = 1.03E-02) (Fig. 2A). In addition, pathway analysis indicated that these specifically and differentially expressed proteins play multiple and significant roles in energy and substance metabolism, such as "Carbon metabolism" (P = 8.63E-03), "Oxidative phosphorylation" (P = 9.52E-03) and "Alanine, aspartate and glutamate metabolism" (P = 4.51E-02) (Fig. 2B). Accordingly, we can reasonably infer that proteins can promote the division and proliferation of muscle cells by regulating substance metabolism and energy distribution, further accelerating the growth rate of TCC. PPI network reconstruction showed that DEPs including *NDUFS1*, *ENO1A*, *NDR2* and *CS* are mainly enriched in "oxidative phosphorylation" (P = 4.13E-04) and "arginine and proline metabolism" (P = 4.12E-02). Meanwhile, species-specific proteins are involved in the "mitochondrial membrane part" (P = 8.46E-03) and "troponin complex" (P = 4.06E-03) (Fig. 3). From a holistic perspective, the results demonstrate that significant proteins could mediate the growth heterosis of TCC in a synergetic way by leading a biased transport, distribution and utilization of substrates and energy. The novel findings are consistent with the characteristics of infertility and fast growth in TCC.

3.5. Species-specific proteins and DEPs play important roles in innate immune pathways

To gain a deeper understanding of the potential molecular basis of the triploid's immune system, as well as its strong resistance, pathway analysis based on the obtained DEPs was conducted. These regulated proteins are involved in the "biosynthesis of antibiotics" (p = 6.66E-04) and "biosynthetic amino acids" (p = 6.33E-04) (Table 4). The synthesis of amino acids is closely related to the synthesis of proteins and low molecules, and the metabolism of amino acids plays a crucial role in maintaining the growth, development and immunity of animals. Our results show that nine proteins (*ENO1A*, *MDH1AA*, *GOT1*, *GOT2A*, *IDH1*, *IDH2*, *IDH3B*, *DLAT*, *SDHA*) (Table 4) play crucial roles in these mentioned pathways. Fig. 4 shows the expression level changes of these proteins in different fishes including TCC, RCC, CC and 4nAT. The results indicated that these proteins were significantly up-regulated in triploid. Significant DEPs could regulate the synthesis and transformation of antibiotics and antimicrobial peptides in a synergetic way. PPI network analysis indicated that *ENO1A* played important regulatory roles in the immune regulation of triploids by involving the migration of monocytes to sites of inflammation or infection as part of the response to pathogens in fish[37] (Fig. 5 and 6).

4. Discussion

TCC is not only a valuable biological material for fish hybridization research, but also an important economic fish.[38] The

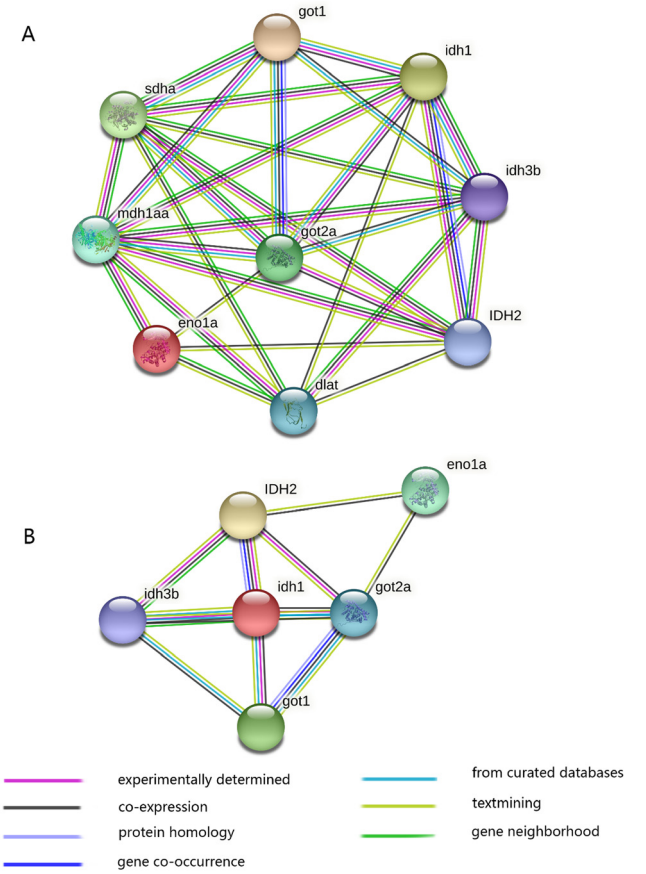

Fig. 5 The network of two pathways. A and B show the results of network reconstruction based on those proteins rich in the "Biosynthesis of antibiotics" and "Biosynthetic amino acids" pathways, respectively. Significant DEPs can regulate the synthesis and transformation of antibiotics and antimicrobial peptides in a synergetic way.

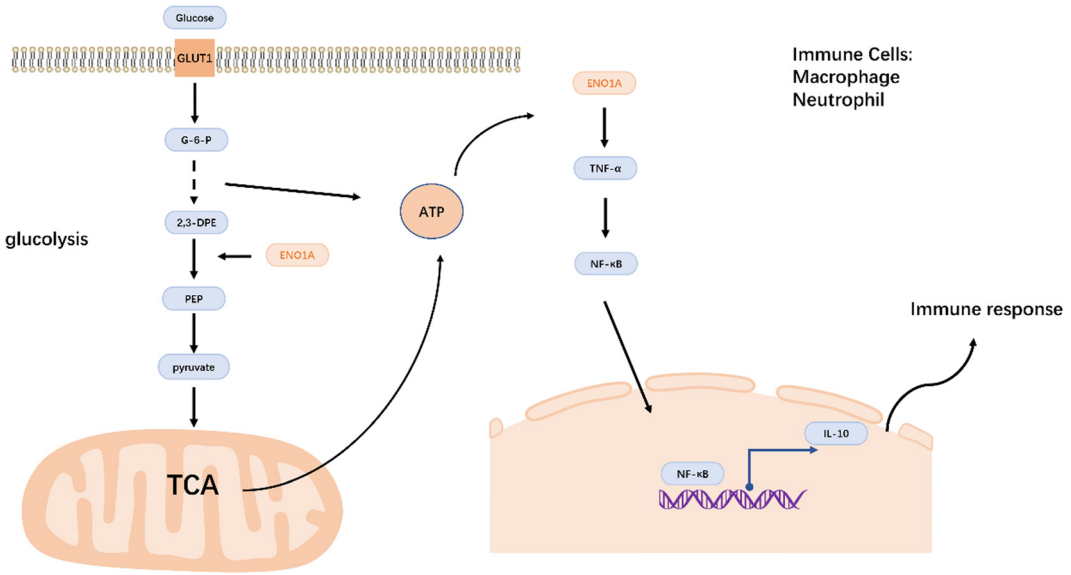

Fig. 6 *ENO1A* is involved in immune mechanisms. *ENO1A* may exist in macrophages and neutrophils and is involved in the regulation of immunization and in inflammatory responses. It also promotes TNF-α expression in order to further promote the production of IL-10. Furthermore, it mediates cytokine production, leukocyte chemotaxis and cell migration.

triploid is highly popular in China because it has an excellent growth rate, tastes delicious and is resistant to viruses. Previous studies on TCC have been mainly focused on its physiological genetics, genome and transcriptome.[39,40] However, there are few studies on the proteomics of TCC. We performed a quantitative proteome on TCC muscle tissue. Muscle tissues are closely related to the growth and development of fish.[41] The results of this study indicate that the DEPs between TCC and its parents are closely related to several species-specific characteristics, such as fast growth, deliciousness and strong immunity.

It has been reported that glutamic acid, glutamine, alanine and aspartic acid are common umami amino acids.[42] In this study, we calculated the amino acid composition and content of the significantly upregulated proteins in TCC. The results show that the top 20 proteins (such as *ENO1A*, *FKBP15B*, *MYH7BB*, *MYHB* and *ATP5F1C*) have a high proportion of umami amino acids (Glu, Gly, Ala, Arg). Glutamic acid plays an important role in the protein metabolism of organisms, participating in many important chemical reactions in animals. It has been reported that the content of L-Glu plays an important role in maintaining the umami taste of food,[43] and the above results have uncovered the reason why TCC tastes more umami than its parents.

Based on the slow development of TCC gonads, it seems likely that some of the energy originally used for gonad development in other fish is diverted into growth, resulting in a faster rate of growth.[3,44] As expected, the DEPs are enriched in terms of cell division, material metabolism and the terms associated with growth, such as ''separation of Sister chromatids''; ''M phase''; ''Cellular responses to stress''; ''Cell Cycle, Mitotic'' and ''RHO GTPase Effectors''. The Rho protein is widely distributed in related immune cells, such as T cells, B cells, NK cells, *etc.* When an organism is infected with microorganisms, the immune inflammatory response is regulated through a series of signal transduction mechanisms, among which, the signal transduction mechanism of the Rho GTPases is one of the most important signaling pathways.[45] During the mitosis and meiosis phases, sister chromatids in all eukaryotic cells separate from each other, resulting in a doubling of cell numbers.[45] These pathways are all related to cell proliferation. To a certain extent, these qualities provide an advantage for triploid growth. KEGG pathways are mainly involved in ''Carbon metabolism, 2-Oxocarboxylic acid metabolism'', ''Oxidative phosphorylation'', ''Biosynthesis of amino acids'', ''Metabolic pathways'', ''Arginine and proline metabolism'' and ''Alanine, aspartate and glutamate metabolism'', and these pathways are closely related to the metabolism of substances and energy. Upregulated proteins enriched in these conditions, as well as pathways, may promote splitting and proliferation in triploid muscle cells, thus accelerating the speed of material energy metabolism.

In addition to head-, kidney-, spleen- and gut-associated lymphoid tissue, muscles are also involved in fish immunity.[19,20] In the results of our study of fish muscle tissue, a series of DEPs, including *ENO1A* and *MDH1AA*, were found to play significant roles in immune-related pathways such as the ''Biosynthesis of antibiotics'' ''Biosynthetic amino acids'' and ''RHO GTPase Effectors''.[46,47] The synthesis and metabolism of amino acids is closely related to animal growth and innate immunity.

5. Conclusion

Triploid fish have excellent characteristics such as fast growth, strong resistance and delicious taste. In this study, we found

that the deliciousness of TCC is mainly related to highly abundant, umami amino acid-enriched proteins, such as *PURBA*, *PVALBI*, *ATP5F1B*, *ATP5FAI* and *DLDH*, and that the species' rapid growth is mainly related to the high expression and regulation of metabolism-related proteins, including *NDUFS1*, *ENO1A* and *CS*, which play significant roles in pathways with respect to the ''generation of precursor metabolites and energy'' and ''arginine and proline metabolism''. Last but not least, we identified several proteins associated with disease resistance, including *ENO1A*, *MDH1AA*, *GOT1*, *IDH1*, *DLAT* and *SDHA*. In conclusion, this study may serve as a significant reference map for similar investigations and shed light on deciphering the molecular and biological functions of significant biomarkers in TCC muscle tissue.

Data availability

The proteomics raw data have been deposited in the ProteomeXchange Consortium *via* the iProX partner repository (**https://www.iprox.cn/**) with the dataset identifiers IPX0003802000.

Author contributions

Yong Zeng and Shaojun Liu contributed to the conceptual framework of the study and experimental design. Lingxiang Wang, Qian Zhou and Rongfang Xu contributed to sample acquisition and preparation. Lingxiang Wang, Xiaoping Dong, Yun Wu and Yong Zeng participated in data collection and data analysis. Lingxiang Wang, Xiaoping Dong Li Ren, Chun Zhang, Min Tao, Kaikun Luo and Yong Zeng wrote and revised the manuscript.

Conflicts of interest

All authors state that they have no conflicts of interest.

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