ITRAQ Proteomic Analysis of Yellow and Black Skin in Jinbian Carp (Cyprinus carpio)

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Abstract: Colors are important phenotypic traits for fitness under natural conditions in vertebrates. Previous studies have reported several functional genes and genetic variations of pigmentation, but the formation mechanisms of various skin coloration remained ambiguous in fish. Jinbian carp, a common carp variant, displays two colors (yellow and black) in the skin, thus, it is a good model for investigating the genetic basis of pigmentation. In the present study, using the Jinbian carp as model, isobaric tags for relative and absolute quantification (ITRAQ) proteomics analysis was performed for yellow and black skin, respectively. The results showed that 467 differentially expressed proteins (DEPs) were identified between the yellow skin and the black skin. Similar to mammals, the up-regulated DEPs in black skin included UV excision repair protein RAD23 homolog A (Rad23a), melanoregulin (mreg), 5,6-dihydroxyindole-2-carboxylic acid oxidase5 (tyrp1) and melanocyte protein PMEL (PMEL), which were mainly grouped into melanogenesis pathway. However, several up-regulated DEPs in yellow skin were mainly enriched in nucleotide metabolism, such as GTPase IMAF family member 5 (GIMAP5), AMP deaminase 1 (AMPD1), adenosylhomocysteinase b (ahcy-b), and pyruvate kinase (PKM). In summary, several candidate proteins and their enrichment pathways for color variation in Jinbian carp were identified, which may be responsible for the formation of different colorations.

Keywords: pigmentation; ITRAQ; Jinbian carp; melanin; pteridine

1. Introduction

Colors are important phenotypic traits for fitness under natural conditions in vertebrates [1]. Skin coloration is the result of diverse pigments synthesized by chromatophores, which is affected by several factors, including environmental, nutritional, physiological, and genetic conditions. Among them, the genetic basis of skin pigmentation is the most fundamental and important factor. Therefore, several studies have been devoted to examine the genetic basis of pigmentation in animals. Previous studies have found that many biological pathways and genes were involved in the pigment synthesis, including melanin and pteridine synthesis pathway [2].

In teleost, several chromatophores have been identified, which played an important role in the formation of variety coloration, including melanophores (melanin granules), xanthophores (pteridine or carotenoid granules), iridophores (guanine), leucophores, and erythrophores (carotenoids and...
pteridine) [3–6]. Using teleost as models, several studies have tried to examine the genetic variation among different colored skin. Haffter et al. observed that dominant mutations in genes could change the pigment pattern in adult zebrafish [7]. However, the key genes and biological processes, which are involved in the combination of different colors, are still ambiguous.

Common carp (Cyprinus carpio L.) was the most widely cultivated freshwater fish in the world. In China, common carp has been farmed for more than 2500 years [8,9]. During the long farming history, common carp has evolved several variants, which was caused by geographic isolation and natural as well as human selection pressures [10]. In China, several variants have also been developed in the regional distribution and cultivation zones over thousands of years. Among them, Jinbian carp is an important variant for paddy-field fish culture in China, which appears with two yellow stripes on both sides of its dorsal fin [11]. Due to its variable colors, Jinbian carp is a good system for studying color pattern polymorphism.

In this study, isobaric tags for relative and absolute quantification (iTRAQ) analysis was used to examine the proteomic variations between yellow skin and black skin in Jinbian carp. The aims of our present study were to: (i) provide an overview of the proteome in yellow skin and black skin; (ii) identify differentially expressed proteins (DEPs) that were possibly involved in yellow coloration; (iii) examine the expression levels of key proteins in the melanin and pteridine pathways between two skin colors.

2. Materials and Methods

2.1. Sample Preparation

The common carp (with black skin only) and the manually selected Jinbian common carp (with yellow and black skin) (Figure 1) were collected from Aquatic Species Introduction and Breeding Center of Guangxi, Nanning, China. The yellow skin (Y) and black skin (B) were collected from the same Jinbian carp. In addition, the black skin in the wild common carp (W) was also collected. All the samples in the cryogenic vials were immediately frozen in liquid nitrogen and stored at −80 °C until further processing. All animal experiments were conducted in accordance with the guidelines and approval of the respective Animal research and Ethics committees of Huazhong Agricultural University (Ethical Approval No. HBAC20091138; Date: 15 November 2009).

![Figure 1. Photographs of the wild common carp (A) and Jinbian carp (B).](image)

2.2. Protein Preparation and iTRAQ Labeling

The skin samples were put into the lysis buffer (8 M urea, 0.3% SDS) with protease inhibitors (Thermo, Rockford, IL, USA), and were then sonicated in ice. The protein samples were reduced with DTT (10 mM) at 56 °C for 1 h, then the IAM (55 mM) were used to alkylate the samples in the darkroom for 1 h. The reduced and alkylated protein mixtures were precipitated by adding 4× volume of chilled acetone at −20 °C overnight. After centrifugation at 4 °C with 30,000×g, the pellet was dissolved in 0.5 M TEAB (Applied Biosystems, Milan, Italy) and sonicated in ice. After centrifugation at 4 °C with 30,000×g, an aliquot of the supernatant was taken to determine the protein concentration with
a 2-D Quant Kit (GE Healthcare). The proteins in the supernatant were kept at −80 °C for further analysis. The proteins were digested with modified trypsin at 37 °C overnight and then labeled with different iTRAQ reagents according to the manufacturer’s instruction. The labeled reagent was dissolved in acetonitrile, mixed with the peptides, and then incubated at room temperature for 2 h. The labeled samples were then mixed, desalted, and vacuum-dried. More details for iTRAQ labeling were provided in our previous studies [12].

2.3. Liquid Chromatography Tandem Mass Spectrometry (LC/LC-MS/MS) Analysis

Each fraction was resuspended with loading buffer (5 mM ammonium formate containing 2% acetonitrile; pH = 10) and separated by high-pH reversed-phase liquid chromatography (RPLC, Acquity Ultra Performance LC; Waters, Milford, MA, USA). The solvent A and solvent B was 2% ACN (pH = 10, adjusted by ammonia) and 80% ACN (pH = 10, adjusted by ammonia), respectively. The gradient elution was performed with 0–30% solvent B for 2–38 min and 30–100% solvent B for 38–40 min on a high-pH RPLC column (C18, 1.7 μm, 2.1 mm × 150 mm; Waters Corporation, Milford, MA, USA). All mass spectrometry proteomics data were deposited in Integrated Proteome Resources (iProX, http://www.iprox.org; project ID: IPX0002447000; ProteomeXchange ID: PXD021293).

2.4. Analysis of the Identified Proteins

By using Proteome Discoverer™ Software 2.1 (Thermo, Rockford, IL, USA), the raw data obtained from LC/LC–MS/MS analysis were processed. The proteins were annotated with the Blast2GO (Gene Ontology) program against the nonredundant protein database (NR; NCBI) (https://www.blast2go.com/). The transcriptome data from black and yellow skin of Jinbian carp as well as black skin of common carp was also used as a reference database for protein annotation. In addition, the differentially expressed proteins (DEPs) were further assigned to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (KEGG; http://www.genome.jp/kegg/). The hypergeometric distribution was used for the GO and KEGG pathway enrichment analysis, and Fisher’s exact test was used for accurate test, and Benjamini was used for the correction of multiple tests. In this analysis, the threshold of significant enrichment of GO function and KEGG pathway of DEPs was p-value < 0.05. The p-value of the significant difference between the samples was calculated by using the t-test in the R language. For identifying significantly up- or down-regulated proteins (p-value < 0.05), the threshold values of Y/B or Y/W ratios were ≥1.50 or ≤0.67 (≥1.5-fold), respectively.

3. Results

3.1. Protein Identification and Quantitation

The iTRAQ analysis revealed that 8064 protein hits were detected in the common carp skin by Proteome Discover (Supplementary Table S1). The error in the matching of the peptide segment detected in the database was below 0.05 Da (Figure 2A). As shown in Figure 2B, the peptide length was around in 9–30 aa, and most of peptide segments were enriched in 9–16 aa length. The numbers of peptides identified into the proteins was exhibited in Figure 2C, which showed that the coverage of most protein sequence was at 1–40%. In addition, the molecular weight of most proteins was 11–150 kDa (Figure 2E).
Figure 2. Identification and analysis of the proteome in yellow skin in Jinbian carp (Y), black skin in Jinbian carp (B) and black skin in wild common carp (W). (A) Distribution of peptide segment matching error. (B) The distribution of peptide length. The x-axis indicates the length of the peptide, and the y-axis indicates the number of peptides of the corresponding length. (C) The coverage distribution of identified proteins. Each fan represents the proportion of a range of coverage. The larger the fan area, the more proteins covered in this range. The number outside the fan indicates the number of proteins covered in this range. (D) The distribution of peptide number. The x-axis indicates the number of peptides covering the protein, and the y-axis indicates the number of proteins. (E) The distribution of protein molecular weight. The x-axis shows the size of the identified protein molecular weight (unit: kilodalton, kDa), and the y-axis represents the number of identified proteins reflecting the corresponding size.

3.2. Identification and GO Classification of the Proteins in Common Carp Skin

The annotated proteins were enriched in three GO categories, including biological function, molecular function, and cellular component (Figure 3A). In the molecular functions, most proteins in
common carp skins were enriched in the binding (511 proteins), catalytic (336), structural molecule (85), transporter (38), and antioxidant activities (seven). In the cellular component, common carp skin proteins were enriched in the categories of cell part (478 proteins), protein-containing complex (287), organelle part (234), membrane part (94), extracellular region part (45), supramolecular complex (30), and membrane-enclosed lumen (two). The most represented biological functions included the categories of cellular process (426 proteins), metabolic process (267), and biological regulation (241). Other important biological function allocations included the developmental process (145 proteins), cellular component organization (137), localization (126), response to stimulus (84), and multicellular organismal process (62).

Total of 2193 proteins were enriched in 159 pathways. The top 20 pathway ontologies were shown in Figure 3B. Proteins were most enriched in relation to focal adhesion (193 proteins), endocytosis (168), regulation of actin cytoskeleton (149), tight junction (134), protein processing in endoplasmic reticulum (124), spliceosome (123), MAPK signaling pathway (119), RNA transport (114), Carbon metabolism (114), phagosome (109), necroptosis (98), oxidative phosphorylation (97), adrenergic signaling in cardiomyocytes (95), ribosome (92), apoptosis (84), lysosome (79), salmonella infection (78), insulin signaling pathway (77), ECM-receptor interaction (75), and mTOR signaling pathway (71).

3.3. Identification of Differentially Expressed Proteins (DEPs) between Yellow and Black Skin in Jinbian Carp

A total of 467 DEPs were identified between yellow (Y) and black (B) skin derived from the same Jinbian carp (FC ≥ 1.2 and p-value < 0.05) (Figure 4A), including 94 up-regulated proteins (Table 1) and 373 down-regulated proteins (Table 2) in yellow skin. GO analysis showed that most up-regulated DEPs in yellow skin were preferentially involved in metabolic process, transporter activity, catalytic activity, membrane part, biological regulation, organelle, cellular process, and binding (Figure 5A). Additionally, most down-regulated DEPs in yellow skin compared to black skin were mainly grouped into biological regulation, transporter activity, organelle part, protein-containing
complex, cellular component biogenesis, binding, cellular process, metabolic process, organelle, catalytic activity, and cell part (Figure 5B).

Table 1. The up-regulated differentially expressed proteins (DEPs) in black skin (B) compared to yellow skin (Y).

| Uniprot_ID       | Gene   | Description                                                                 | FC(B/Y) | p Value       |
|------------------|--------|----------------------------------------------------------------------------|---------|---------------|
| MLRB_CHICK       | N/A    | Myosin regulatory light chain 2B                                           | 6.34    | 0.0029041     |
| MYL4_HUMAN       | MYL4   | Myosin light chain 4                                                        | 5.22    | 0.0035055     |
| TNN2_CHICK       | TNN2   | Troponin T                                                                  | 4.44    | 0.0005641     |
| MYPC3_CHICK      | MYBPC3 | Myosin-binding protein C                                                    | 4.16    | 7.544 × 10⁻⁵  |
| TNN3_HUMAN       | TNN3   | Troponin T                                                                  | 4.01    | 0.0049163     |
| TNNI1_HUMAN      | TNNI1  | Troponin I                                                                  | 3.53    | 2.086 × 10⁻⁵  |
| MYH7_HUMAN       | MYH7   | Myosin-7                                                                    | 3.53    | 0.0402934     |
| AT2A1_MAKNI      | atp2a1 | Sarcoplasmic/endoplasmic reticulum calcium ATPase 1                         | 3.28    | 0.0028990     |
| TPM1_LIZAU       | N/A    | Tropomyosin alpha-1 chain                                                   | 3.27    | 0.0002662     |
| TNN3_XENLA       | tnn3   | Troponin I                                                                  | 3.22    | 0.0001500     |
| COX42_THUOB      | N/A    | Cytochrome c oxidase subunit 4 isofrom 2                                   | 3.11    | 0.0014771     |
| TNN1_MOUSE       | Tnn1   | Troponin I                                                                  | 3.03    | 0.0042539     |
| ACTN4_CHICK      | ACTN4  | Alpha-actinin-4                                                             | 2.82    | 0.0037840     |
| ACTN2_CHICK      | ACTN2  | Alpha-actinin-2                                                             | 2.75    | 0.0009886     |
| HHATL_HUMAN      | HHATL  | Protein-cysteine N-palmitoyltransferase HHAT-like protein                   | 2.71    | 0.0062292     |
| MYPC1_HUMAN      | MYBPC1 | Myosin-binding protein C                                                    | 2.61    | 0.0433731     |
| NNTM_HUMAN       | NNT    | NAD(P) transhydrogenase                                                     | 2.50    | 0.0009916     |
| KCRB_CHICK       | CKB    | Creatine kinase B-type                                                      | 2.48    | 0.0016669     |
| PYGM_MACFA       | PYGM   | Glycogen phosphorylase                                                      | 2.36    | 0.0038506     |
| IDHP_MACFA       | IDH2   | Isocitrate dehydrogenase [NADP]                                             | 2.34    | 0.0043346     |
| AT2A2_CHICK      | ATP2A2 | Sarcoplasmic/endoplasmic reticulum calcium ATPase 2                         | 2.30    | 0.0008799     |
| ACTC_RAT         | Actc1  | Actin                                                                       | 2.27    | 0.0071161     |
| AATM_RAT         | Got2   | Aspartate aminotransferase                                                  | 2.25    | 0.0006509     |
| MYH7_PIG         | MYH7   | Myosin-7                                                                    | 2.22    | 0.0386928     |
| UCRI_AOTAZ       | UQCRFS1| Cytochrome b-c1 complex subunit Rieske                                      | 2.15    | 0.0031625     |
| SRCA_MOUSE       | Srl    | Sarcalumenin                                                                | 2.09    | 0.0002984     |
| NEBU_HUMAN       | NEB    | Nebulin                                                                     | 1.96    | 0.0014726     |
| ECHA_MOUSE       | Hadha  | Trifunctional enzyme subunit alpha                                          | 1.96    | 0.0102789     |
| NDUFA4_DANRE     | ndufa4 | Cytochrome c oxidase subunit NDUFA4                                         | 1.96    | 0.0069833     |
| MYH6_MOUSE       | Myh6   | Myosin-6                                                                    | 1.93    | 0.0204875     |
| MYH7_MOUSE       | Myh7   | Myosin-7                                                                    | 1.91    | 0.0294791     |
| COX5A_MOUSE      | Cox5a  | Cytochrome c oxidase subunit 5A                                             | 1.90    | 0.0003158     |
| MYH7_HORSE       | MYH7   | Myosin-7                                                                    | 1.84    | 0.0194304     |
| MDHM_FRAAN       | MMDHI  | Malate dehydrogenase                                                        | 1.82    | 0.0010926     |
Table 1. Cont.

| Uniprot_ID       | Gene       | Description                                | FC(B/Y) | p Value     |
|------------------|------------|--------------------------------------------|---------|-------------|
| GLYM_RABIT       | SHMT2      | Serine hydroxymethyltransferase            | 1.82    | 0.0453498   |
| DESM_PIG         | Des        | Desmin                                     | 1.79    | 0.0065272   |
| ATPA_PIG         | ATP5F1A    | ATP synthase subunit alpha                 | 1.78    | 0.0011789   |
| ATP5F1_PONAB     | ATP5F1     | ATP synthase-coupling factor 6             | 1.77    | 0.0148907   |
| ACSL1_CAVPO      | ACSL1      | Long-chain-fatty-acid-CoA ligase 1         | 1.75    | 0.0038413   |
| MYO6_CHICK       | MYO6       | Unconventional myosin-VI                  | 1.75    | 0.0009482   |
| CISY_DANRE       | cs         | Citrate synthase                           | 1.73    | 0.008091    |
| KPYM_CHICK       | PKM        | Pyruvate kinase PKM                        | 1.71    | 0.0122511   |
| UCR1_MOUSE       | Uqcrfs1    | Cytochrome b-c1 complex subunit Rieske     | 1.70    | 0.0031496   |
| ACADM_RAT        | Acadm      | Medium-chain specific acyl-CoA dehydrogenase | 1.70  | 0.0438522   |
| SYPH_MOUSE       | Syp        | Synaptophysin                              | 1.70    | 0.0041077   |
| ATSF1_PONAB      | ATP5F1     | ATP synthase F(0) complex subunit B1       | 1.69    | 0.0064455   |
| ADT3_BOVIN       | SLC25A6    | ADP/ATP translocase 3                      | 1.69    | 0.0150770   |
| NLS1B_DANRE      | mfsd2ab    | Sodium-dependent lysophosphatidylcholine symporter 1-B | 1.68 | 0.0272424   |
| ECHA_RAT         | Hadha      | Trifunctional enzyme subunit alpha         | 1.67    | 0.0027369   |
| ADT2_BOVIN       | SLC25A5    | ADP/ATP translocase 2                      | 1.66    | 0.0289562   |
| ACON_BOVIN       | ACO2       | Aconitate hydratase                        | 1.66    | 0.0006630   |
| KCNV1_BOVIN      | KCNV1      | Potassium voltage-gated channel subfamily V member 1 | 1.65 | 0.0236049   |
| PGAM2_HUMAN      | PGAM2      | Phosphoglycerate mutase 2                  | 1.64    | 0.0458743   |
| ECHB_MACFA       | HADHB      | Trifunctional enzyme subunit beta          | 1.64    | 0.0153694   |
| NDUAA_HUMAN      | NDUFA10    | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10 | 1.64 | 0.0027091   |
| ADT1_BOVIN       | SLC25A4    | ADP/ATP translocase 1                      | 1.64    | 0.0373976   |
| NDUS4_HUMAN      | NDUIFS4    | NADH dehydrogenase [ubiquinone] iron-sulfur protein 4 | 1.61 | $1.724 \times 10^{-5}$ |
| MYOZ2_PONAB      | MYOZ2      | Myozenin-2                                 | 1.60    | 0.0020216   |

Note: N/A: Not applicable; FC: fold changes.

Table 2. The up-regulated DEPs in yellow skin (Y) compared to black skin (B).

| Uniprot_ID       | Gene   | Description                                | FC(Y/B) | p Value     |
|------------------|--------|--------------------------------------------|---------|-------------|
| FXR2_HUMAN       | FXR2   | Fragile X mental retardation syndrome-related protein 2 | 5.18    | 0.0018055   |
| MYP2C_MOUSE      | Mypc2  | Myosin-binding protein C                   | 3.99    | 0.001018    |
| TPM1_LIZAU       | N/A    | Tropomyosin alpha-1 chain                  | 3.21    | 0.0026475   |
| PRV7_DANRE       | pralb7 | Parvalbumin-7                              | 2.86    | 0.0203993   |
| TPM1_XENLA       | tpm1   | Tropomyosin alpha-1 chain                  | 2.57    | 0.0042248   |
| CP110_HUMAN      | CCP110 | Centriolar coiled-coil protein of 110 kDa  | 2.45    | 0.0203727   |
| LEG12_HUMAN      | LGALS12 | Galectin-12                              | 1.73    | 0.0051317   |
### Table 2. Cont.

| Uniprot_ID       | Gene     | Description                  | FC(Y/B) | p Value  |
|------------------|----------|------------------------------|---------|----------|
| H11_HUMAN        | HIST1H1A | Histone H1.1                 | 1.68    | 0.0206282|
| COCA1_MOUSE      | Col12a1  | Collagen alpha-1(XII) chain  | 1.67    | 0.0363538|
| LEG4_BOVIN       | LGALS4   | Galectin-4                   | 1.66    | 0.0101980|
| TITIN_MOUSE      | Ttn      | Titin                        | 1.62    | 0.0323899|
| TITIN_HUMAN      | TTN      | Titin                        | 1.62    | 0.0486235|
| RHOG_HUMAN       | RHOG     | Rho-related GTP-binding protein RhoG | 1.59 | 0.0213023|
| COCA1_HUMAN      | COL12A1  | Collagen alpha-1(XII) chain  | 1.56    | 0.0135574|
| CAHZ_DANRE       | cahz     | Carbonic anhydrase            | 1.45    | 0.0387359|
| C2D1B_XENLA      | cc2d1b   | Coiled-coil and C2 domain-containing protein 1B | 1.43 | 0.0146113|
| PLPP1_CAPO       | PLPP1    | Phospholipid phosphatase 1    | 1.42    | 0.0265325|
| EIF1B_HUMAN      | EIF1B    | Eukaryotic translation initiation factor 1b | 1.42 | 0.0201610|
| BRE1A_HUMAN      | RNF20    | E3 ubiquitin-protein ligase BRE1A | 1.40 | 0.0237980|
| HCE1_ORYLA       | hcea     | High choriolytic enzyme 1     | 1.35    | 0.0377692|
| SPTB2_MOUSE      | Sptbn1   | Spectrin beta chain           | 1.34    | 0.0234210|
| HBB1_DANRE       | ba1      | Hemoglobin subunit beta-1     | 1.34    | 0.0356035|
| B3AT_ONCMY       | slc4a1   | Band 3 anion exchange protein  | 1.33    | 0.0495074|
| DMD_PIG          | DMD      | Dystrophin                    | 1.33    | 0.0089534|
| AQP3_MOUSE       | Aqp3     | Aquaporin-3                   | 1.32    | 0.0099533|
| H3_DROME         | His3     | Histone H3.2                  | 1.32    | 0.0314604|
| MCM7_XENTR       | mcm7     | DNA replication licensing factor mcm7 | 1.32 | 0.0348232|
| H2B12_XENLA      | N/A      | Histone H2B 1.2               | 1.31    | 0.0439002|
| NLRC3_HUMAN      | NLRC3    | Protein NLRC3                 | 1.31    | 0.0217364|
| GOLP3_MOUSE      | Golph3   | Golgi phosphoprotein 3        | 1.30    | 0.0116354|
| TSP1_XENLA       | tibs1    | Thrombospondin-1              | 1.30    | 0.0151795|
| PSA2_CARAU       | psma2    | Proteasome subunit alpha type-2 | 1.29 | 0.0222874|
| FKBP5_HUMAN      | FKBP5    | Peptidyl-prolyl cis-trans isomerase FKBP5 | 1.29 | 0.0071276|
| FLNC_RAT         | Flnc     | Filamin-C                     | 1.29    | 0.0458236|
| RALB_MACFA       | RALB     | Ras-related protein Ral-B     | 1.26    | 0.0165614|
| CP3AA_MESAU      | CYP3A10  | Lithocholate 6-beta-hydroxylase | 1.26 | 0.0305838|
| CD63_MOUSE       | Cd63     | CD63 antigen                  | 1.25    | 0.0002011|
| ZYX_CHICK        | ZYX      | Zyxin                         | 1.24    | 0.0092652|
| CATL_SARPE       | N/A      | Cathepsin L                   | 1.24    | 0.0087340|
| PTX3_MOUSE       | Ptx3     | Pentraxin-related protein PTX3 | 1.23 | 0.0302834|
| CATZ_RAT         | Ctsz     | Cathepsin Z                   | 1.23    | 0.0072279|
| DYH1C1_MOUSE     | Dync1h1  | Cytoplasmic dynein 1 heavy chain 1 | 1.23 | 0.0327325|
| AR6P6_BOVIN      | ARL6IP6  | ADP-ribosylation factor-like protein 6-interacting protein 6 | 1.23 | 0.0445232|
| TPM3_MOUSE       | Tpm3     | Tropomyosin alpha-3 chain     | 1.23    | 0.0293024|
| TBD2B_MOUSE      | Tbc1d2b  | TBC1 domain family member 2B  | 1.22    | 0.0450312|
| NEBU_HUMAN       | NEB      | Nebulin                       | 1.22    | 0.0288621|
In addition to the GO analysis, KEGG pathway enrichment analysis was also used to further elucidate the putative functions of these DEPs. These results demonstrated that down-regulated proteins in yellow skin were mainly involved in the oxidative phosphorylation (53/142), carbon metabolism (33/142), cardiac muscle contraction (22/142), biosynthesis of amino acids (14), fatty acid metabolism (13), calcium signaling pathway (13), fatty acid degradation (13), citrate cycle (13), necroptosis (12), propanoate metabolism (12), valine, leucine and isoleucine degradation (12), glycolysis/gluconeogenesis (11), adrenergic signaling in cardiomyocytes (11), cysteine and methionine metabolism (10), purine metabolism (two), biosynthesis of unsaturated fatty acids (8), beta-Alanine metabolism (eight), butanoate metabolism (eight), tryptophan metabolism (eight), cellular senescence (eight), lysine degradation (eight), starch and sucrose metabolism (eight), and fatty acid elongation (eight) (Figure 6A). In addition, the up-regulated proteins in yellow skin were enriched in the phagosome (5/21), lysosome (five), focal adhesion (three), salmonella infection (three), adrenergic signaling in cardiomyocytes (three), cardiac muscle contraction (three), apoptosis (three), mitophagy (two), and MAPK signaling pathway (one) (Figure 6A).

**Table 2. Cont.**

| Uniprot_ID       | Gene         | Description                      | FC(Y/B) | p Value     |
|------------------|--------------|----------------------------------|---------|-------------|
| LSM3_MOUSE       | Lsm3         | U6 snRNA-associated Sm-like protein LSm3 | 1.21    | 0.0099663   |
| TXB1B_DANRE      | tax1bp1b     | Tax1-binding protein 1 homolog B  | 1.21    | 0.0473674   |
| HEPH_MOUSE       | Heph         | Hephaestin                       | 1.21    | 0.0252919   |
| CAD26_HUMAN      | CDH26        | Cadherin-like protein 26         | 1.21    | 0.0214089   |
| NIBL1_HUMAN      | FAM129B      | Niban-like protein 1             | 1.21    | 0.0188033   |
| CNN1_MUSPF       | CNN1         | Calponin-1                       | 1.21    | 0.0017860   |

**Figure 4.** Volcano plot of DEPs in B, Y, and W. (A) Volcano plot of DEPs in B and Y. (B) Volcano plot of DEPs in W and Y. Splashes represent different genes, Yellow dots indicate proteins that are significantly up-regulated at $p < 0.05$, red dots indicate proteins that are significantly up-regulated at $p < 0.01$, light blue dots indicate proteins that are significantly down-regulated at $p < 0.05$, blue dots indicate protein that are significantly down-regulated under $p < 0.01$ conditions, and black spots are non-significantly differentially expressed proteins.
Figure 5. Gene ontology (GO) enrichment analysis of DEPs among Y, B, and W. Statistics of top 15 enriched GO terms for DEPs in yellow skin (A) and black skin (B) in Jinbian carp; Statistics of top 15 enriched GO terms for DEPs in Y (C) and W (D); p < 0.001 is marked as "***", p < 0.01 is marked as "**", and p < 0.05 is marked as "*".
3.4. DEP Analysis between Yellow Skin in Jinbian Carp (Y) and Black Skin in Wild Common Carp (W)

A total of 129 DEPs were also identified between Y and W (FC ≥ 1.2 and p-value < 0.05 (Figure 4B), among which included 58 up-regulated proteins (Table 3) and 71 down-regulated proteins in Y (Table 4). GO analysis showed that most up-regulated genes in Y were grouped into biological regulation, virion part, structural molecule activity, binding, cellular process, developmental process, organelle part, localization, catalytic activity, protein-containing complex, cellular component biogenesis, cell part, metabolic process, organelle, and multicellular organismal process (Figure 5C). In addition, most down-regulated DEPs in Y compared to W were grouped into cellular process, immune system process, cell part, developmental process, extracellular region, organelle, protein-containing complex, localization, membrane part, and binding (Figure 5D).

KEGG analysis showed that up-regulated genes in Y compared to W were preferentially grouped into lysosome (22/84), apoptosis (11/84), tight junction (10), glycolysis/gluconeogenesis (nine), phagosome (eight), biosynthesis of amino acids (seven), carbon metabolism (seven), pentose phosphate pathway (six), focal adhesion (five), regulation of actin cytoskeleton (five), starch and sucrose metabolism (five), fructose and mannose metabolism (five), salmonella infection (four), galactose metabolism (four), glycosaminoglycan degradation (four), adrenergic signaling in cardiomyocytes...
(four), calcium signaling pathway (four), cardiac muscle contraction (four), arginine and proline metabolism (four), and MAPK signaling pathway (three) (Figure 6B). In addition, the down-regulated genes in Y compared to W were mainly grouped into spliceosome (49/354), focal adhesion (48), RNA transport (31), ECM-receptor interaction (31), regulation of actin cytoskeleton (24), endocytosis (22), mRNA surveillance pathway (19), protein processing in endoplasmic (17), reticulum (15), phagosome (14), ribosome biogenesis in eukaryotes (14), insulin signaling pathway (14), apoptosis (13), necroptosis (12), adrenergic signaling in cardiomyocytes (11), carbon metabolism (11), melanogenesis (10), and apelin signaling pathway (10) (Figure 6B).

Table 3. The DEPs up-regulated in W compared to Y (FC ≥ 1.6).

| Uniprot_ID     | Gene       | Description                                           | FC(W/Y) | p Value            |
|----------------|------------|-------------------------------------------------------|---------|--------------------|
| HIBCH_DANRE    | hibch      | 3-hydroxyisobutyryl-CoA hydrolase                      | 4.52    | 0.0106552          |
| RD23A_MOUSE    | Rad23a     | UV excision repair protein RAD23 homolog A             | 3.47    | 0.0130752          |
| MREG_DANRE     | mreg       | Melanoregulin                                         | 3.20    | 3.6735 × 10⁻⁵      |
| LAMB1_HUMAN    | LAMB1      | Laminin subunit beta-1                                | 3.18    | 0.0192734          |
| FBXL7_MOUSE    | Fbxl7      | F-box/LRR-repeat protein 7                            | 2.48    | 0.0064965          |
| MX2_ONCMY      | mx2        | Interferon-induced GTP-binding protein Mx2             | 2.43    | 0.0299254          |
| K1C1_CARAU     | N/A        | Keratin                                               | 2.36    | 0.0008656          |
| ST2B1_MOUSE    | Sult2b1    | Sulfotransferase family cytosolic 2B member 1          | 2.27    | 0.0020935          |
| TBCB_BOVIN     | TBCB       | Tubulin-folding cofactor B                            | 2.26    | 0.0141326          |
| CMYA5_HUMAN    | CMYA5      | Cardiomyopathy-associated protein 5                    | 2.17    | 0.0476189          |
| NF1_COTJA      | NEFL       | Neurofilament light polypeptide                        | 2.14    | 0.0275144          |
| ETFA_HUMAN     | ETFA       | Electron transfer flavoprotein subunit alpha           | 1.97    | 0.0429857          |
| MYBPC3_CHICK   | MYBPC3     | Myosin-binding protein C                               | 1.92    | 0.0243995          |
| RDH12_HUMAN    | RDH12      | Retinol dehydrogenase 12                              | 1.92    | 0.0340642          |
| CWC22_DANRE    | cwc22      | Pre-mRNA-splicing factor CWC22 homolog                 | 1.89    | 0.0230742          |
| AT2A1_MAKNI    | atp2a1     | Sarcomplasmic/endoplasmic reticulum calcium ATPase 1   | 1.85    | 0.0471695          |
| PLEC_MOUSE     | Plec       | Plectin                                               | 1.84    | 0.0021985          |
| GSE1_DANRE     | gse1       | Genetic suppressor element 1                          | 1.83    | 0.0457959          |
| PLEC_HUMAN     | PLEC       | Plectin                                               | 1.81    | 0.0268665          |
| OPA1_DANRE     | opa1       | Dynamin-like 120 kDa protein                          | 1.81    | 0.0430209          |
| LAMA4_HUMAN    | LAMA4      | Laminin subunit alpha-4                               | 1.81    | 0.0229941          |
| DCXR_MESAU     | DCXR       | L-xylulose reductase                                  | 1.81    | 0.0028180          |
| A2MP_MOUSE     | A2m        | Alpha-2-macroglobulin-P                               | 1.80    | 0.0145305          |
| MF2NB_DANRE    | borcs8     | Protein MEF2BNB                                        | 1.80    | 0.0487061          |
| SYK_HUMAN      | KARS       | Lysine—tRNA ligase                                    | 1.80    | 0.0010683          |
| SPAG7_DANRE    | spag7      | Sperm-associated antigen 7 homolog                    | 1.76    | 0.0268045          |
| COX42_THUOB    | N/A        | Cytochrome c oxidase subunit 4 isoform 2               | 1.76    | 0.0198968          |
Table 3. Cont.

| Uniprot_ID     | Gene  | Description                                      | FC(W/Y) | p Value    |
|----------------|-------|--------------------------------------------------|---------|------------|
| AT2A1_RABIT    | ATP2A1| Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 | 1.75    | 0.0471735  |
| NUP98_RAT      | Nup98 | Nuclear pore complex protein Nup98-Nup96          | 1.75    | 0.0376341  |
| ALKB5_DANRE    | alkb5 | RNA demethylase ALKBH5                            | 1.73    | 0.0135027  |
| CLIC2_HUMAN    | CLIC2 | Chloride intracellular channel protein 2          | 1.72    | 0.0347927  |
| TYRP1_CARAU    | tyrp1 | 5,6-dihydroxyindole-2-carboxylic acid oxidase5    | 1.72    | 0.0002676  |
| RBP1_HUMAN     | RBP1  | Ribosome-binding protein 1                        | 1.71    | 0.0366481  |
| SRSF5_HUMAN    | SRSF5 | Serine/arginine-rich splicing factor 5             | 1.70    | 0.0002839  |
| COCA1_CHICK    | COL12A1| Collagen alpha-1(XII) chain                      | 1.70    | 0.0262727  |
| AGRB2_MOUSE    | Adgrb2| Adhesion G protein-coupled receptor B2            | 1.70    | 0.0044674  |
| LPIN1_HUMAN    | LPIN1 | Phosphatidate phosphatase LPIN1                   | 1.69    | 0.0197062  |
| PLIN5_RAT      | Plin5  | Perilipin-5                                       | 1.69    | 0.0165470  |
| CALD1_MELGA    | CALD1 | Caldesmon                                        | 1.67    | 0.0016593  |
| PMEL_CHICK     | PMEL  | Melanocyte protein PMEL                           | 1.67    | 0.0316520  |
| RM43_BOVIN     | MRPL43| 39S ribosomal protein L43                         | 1.67    | 0.0311367  |
| AIP_RAT        | Aip   | AH receptor-interacting protein                   | 1.66    | 0.0455057  |
| MRP_BOVIN      | MARCKS1| MARCKS-related protein                            | 1.65    | 0.0044254  |
| PSMF1_PONAB     | PSMF1 | Proteasome inhibitor PI31 subunit                 | 1.65    | 0.0351891  |
| PTX3_HUMAN     | PTX3  | Pentraxin-related protein PTX3                    | 1.64    | 0.0357322  |
| HSPE7_DANRE    | hsps14| Heat shock 70 kDa protein 14                      | 1.64    | 0.0100857  |
| K2C8_HUMAN     | KRT8  | Keratin                                          | 1.64    | 0.0073882  |
| TPDS4_HUMAN    | TPDS52L2| Tumor protein D54                                | 1.63    | 0.0281041  |
| PCS2_BOVIN     | DCN   | Decorin                                          | 1.63    | 0.0046897  |
| TR111_RAT      | Trim11| E3 ubiquitin-protein ligase TRIM11                | 1.63    | 0.0092775  |
| RFP2_BOVIN     | RPF2  | Ribosome production factor 2 homolog             | 1.63    | 0.0380427  |
| ECHD1_DANRE    | echdc1| Ethylmalonyl-CoA decarboxylase                    | 1.63    | 0.0064541  |
| NAP8_HUMAN     | NAP8  | Beta-soluble NSF attachment protein               | 1.62    | 0.0255251  |
| PKP3_MOUSE     | Pkp3  | Plakophilin-3                                    | 1.61    | 0.0044704  |
| SSF1_MOUSE     | Ppan  | Suppressor of SWI4 1 homolog                     | 1.61    | 0.0288496  |
| ADDG_HUMAN     | ADD3  | Gamma-adducin                                    | 1.61    | 0.0010349  |
| PBX1_HUMAN     | PBX1  | Pre-B-cell leukemia transcription factor 1        | 1.60    | 0.0001923  |
| RTN1_PANTR     | RTN1  | Reticulon-1                                      | 1.60    | 0.0389269  |

Note: N/A: Not applicable.
Table 4. The DEPs up-regulated in Y compared to W (FC $\geq 1.5$).

| Uniprot_ID         | Gene     | Description                                              | FC(Y/W) | p Value (W/Y) |
|--------------------|----------|----------------------------------------------------------|---------|---------------|
| MYOM2_HUMAN        | MYOM2    | Myomesin-2                                               | 4.87    | 0.0034066     |
| DDR2_MOUSE         | Ddr2     | Discoidin domain-containing receptor 2                   | 4.68    | 0.0283762     |
| TNCC2_ANGAN        | N/A      | Troponin C                                               | 4.50    | 0.0072123     |
| MYOZ1_HUMAN        | MYOZ1    | Myozenin-1                                               | 4.01    | 0.0065529     |
| MYSS_CYPACA        | N/A      | Myosin heavy chain                                       | 3.53    | 0.0017226     |
| PRV2_DANRE         | pvalb2   | Parvalbumin-2                                            | 3.34    | 0.0176125     |
| MYOZ1_BOVIN        | MYOZ1    | Myozenin-1                                               | 3.25    | 0.0011707     |
| ACTN3_BOVIN        | ACTN3    | Alpha-actinin-3                                          | 3.09    | 0.0014259     |
| PDL7_BOVIN         | PDLIM7   | PDZ and LIM domain protein 7                             | 3.05    | 0.0062208     |
| SAHHB_XENLA        | ahcy-b   | Adenosylhomocysteinase B                                 | 3.01    | 0.0097743     |
| TITIN_HUMAN        | TTN      | Titin                                                   | 2.97    | 0.0040666     |
| DYST_HUMAN         | DST      | Dystonin                                                | 2.90    | 0.0213198     |
| MYBP_CHEICK        | MYBP     | Myosin-binding protein H                                 | 2.83    | 0.0015750     |
| CASR_BOVIN         | CASR     | Extracellular calcium-sensing receptor                    | 2.81    | 0.0064296     |
| NRG_DROME          | Nrg      | Neuroglian                                              | 2.75    | 0.0083956     |
| MTUS2_HUMAN        | MTUS2    | Microtubule-associated tumor suppressor candidate 2      | 2.74    | 0.0214764     |
| MYPC2_MOUSE        | Mybpc2   | Myosin-binding protein C                                 | 2.72    | 0.0059417     |
| AMPD1_HUMAN        | AMPD1    | AMP deaminase 1                                          | 2.61    | 0.007214      |
| MAP7_CHEICK        | MAP7     | Enscinsin                                               | 2.61    | 0.0064867     |
| TNNT3_COTJA        | TNNT3    | Troponin T                                              | 2.60 $\times 10^{-5}$ | 8.4696 \times 10^{-5} |
| GIMAP5_HUMAN       | GIMAP5   | GTPase IMAP family member 5                              | 2.29    | 0.0243491     |
| AOXC_MOUSE         | Aox3     | Aldehyde oxidase 3                                        | 2.22    | 0.0150366     |
| KIC14_MOUSE        | Kr114    | Keratin                                                 | 2.17    | 0.0462584     |
| TNIN2_RABIT        | TNIN2    | Troponin I                                              | 2.16    | 0.0005866     |
| KPYM_CHEICK        | PKM      | Pyruvate kinase PKM                                      | 2.16    | 0.0370822     |
| MPSF_CHEICK        | N/A      | M-protein                                               | 2.13    | 0.0065892     |
| KCRM_CANFA         | CKM      | Creatine kinase M-type                                   | 2.11    | 0.0154615     |
| LDB3_HUMAN         | LDB3     | LIM domain-binding protein 3                             | 2.10    | 0.0389130     |
| FUC0_HUMAN         | FUC0     | Tissue alpha-L-fucosidase                                | 2.06    | 0.0105965     |
| MYO1F_HUMAN        | MYO1F    | Unconventional myosin-If                                 | 2.04    | 0.0272882     |
| PRKCD_HUMAN        | PRKCD    | Protein kinase C delta type                              | 2.01    | 0.0146456     |
| FLNC_RAT           | Flnc     | Filamin-C                                               | 2.01    | 0.0345344     |
| TPM3_HUMAN         | TPM3     | Tropomyosin alpha-3 chain                                | 1.99    | 0.0062754     |
| RNT2_DANRE         | ruset2   | Ribonuclease T2                                          | 1.99    | 0.007026      |
| CHP3_XENLA         | tesc     | Calcineurin B homologous protein 3                       | 1.98    | 0.0098880     |
| VTNCE_RABIT        | VTN      | Vitronectin                                             | 1.97    | 0.0419940     |
| ACTA_TAKRU         | acta1a   | Actin                                                   | 1.94    | 0.0035608     |
| FUC0_MOUSE         | Fuc0     | Tissue alpha-L-fucosidase                                | 1.91    | 0.0197286     |
### Table 4. Cont.

| Uniprot_ID      | Gene    | Description                          | FC(Y/W) | p Value (W/Y) |
|-----------------|---------|--------------------------------------|---------|---------------|
| MYH4_HUMAN      | MYH4    | Myosin-4                             | 1.86    | 0.0243060    |
| IF44L_MOUSE     | Ifi44l  | Interferon-induced protein 44-like   | 1.86    | 0.0264745    |
| BAX_BOVIN       | BAX     | Apoptosis regulator BAX              | 1.84    | 0.0027166    |
| NEBU_HUMAN      | NEB     | Nebulin                              | 1.84    | 0.0041143    |
| AKAP12_HUMAN    | AKAP12  | A-kinase anchor protein 12           | 1.82    | 0.0224758    |
| EEF1A1_HORSE    | EEF1A1  | Elongation factor 1-alpha 1         | 1.81    | 0.0125514    |
| K1C13_ONCMY     | Krt13   | Keratin                              | 1.80    | 0.0162506    |
| FUCM_DANRE      | Fum     | Fucose mutarotase                    | 1.77    | 0.0014529    |
| TIMP3_XENLA     | Timp3   | Metalloproteinase inhibitor 3        | 1.74    | 0.029090    |
| CNIH4_BOVIN     | CNIH4   | Protein cornichon homolog 4          | 1.72    | 0.0420714    |
| PDL17_MOUSE     | Pdlim7  | PDZ and LIM domain protein 7         | 1.70    | 0.0173236    |
| CATB_CHICK      | CTSB    | Cathepsin B                          | 1.69    | 0.0004048    |
| OBSCN_MOUSE     | Obscn   | Obscurin                             | 1.68    | 0.0015550    |
| MYOZ2_PONAB     | MYOZ2   | Myozenin-2                           | 1.66    | 0.0485948    |
| SAP_CHICK       | PSAP    | Prosaposin                           | 1.66    | 0.0389310    |
| PUA1A_SALSA     | Adssl1a | Adenylosuccinate synthetase isozyme 1 A | 1.65  | 0.0115606   |
| CFAB_BOVIN      | CFB     | Complement factor B                  | 1.64    | 0.0096244    |
| LEG12_HUMAN     | LGALS12 | Galectin-12                          | 1.63    | 0.0231777    |
| WDR47_MOUSE     | Wdr47   | WD repeat-containing protein 47      | 1.62    | 0.0030872    |
| ALDOA_MOUSE     | Alloa   | Fructose-bisphosphate aldolase A     | 1.62    | 0.0033610    |
| CFAB_MOUSE      | Cfb     | Complement factor B                  | 1.60    | 0.0027860    |
| G3P_CHICK       | GAPDH   | Glyceraldehyde-3-phosphate dehydrogenase | 1.58  | 0.0436886   |
| DMD_CHICK       | DMD     | Dystrophin                           | 1.56    | 0.0007040    |
| PPGB_MOUSE      | Ctsa    | Lysosomal protective protein          | 1.55    | 0.0207043    |
| DPP2_MOUSE      | Dpp7    | Dipeptidyl peptidase 2              | 1.54    | 0.0001501    |
| TITIN_MOUSE     | Ttn     | Titin                                | 1.54    | 0.0002004    |
| NUPR2_HUMAN     | Nupr2   | Nuclear protein 2                    | 1.53    | 0.0135287    |
| LGMN_BOVIN      | LGMN    | Legumain                             | 1.53    | 0.0159403    |
| BGLR_MOUSE      | Gusb    | Beta-glucuronidase                   | 1.53    | 0.0195223    |
| CATH_PIG        | CTSH    | Pro-cathepsin H                      | 1.52    | 0.0364061    |
| ING5_HUMAN      | ING5    | Inhibitor of growth protein 5        | 1.52    | 0.0011046    |
| ALDOA_SALSA     | N/A     | Fructose-bisphosphate aldolase A     | 1.51    | 0.0039936    |
| CAPZB_CHICK     | CAPZB   | F-actin-capping protein subunit beta isoforms 1 and 2 | 1.51  | 0.0220029   |

**Note**: N/A: Not applicable.

### 4. Discussion

The common carp has been widely farmed in Europe and Asia. In the long-term breeding process, the common carp has evolved hundreds of strains or varieties that display a rich biodiversity and are
diverse in skin colors [13]. Due to their diverse skin colors, the common carp was used as a good model to elucidate the skin pigmentation process. Various fish colorations are determined by the density and position of different pigment cells, which is believed to be mainly controlled by genetic factors. As we know, both the melanin and the pteridine synthesis pathways have been found in teleost. The black pigment, melanin, is generated in melanophores through the melanin synthesis pathway [2], and the pteridine synthesis pathway could produce the yellow or reddish pteridine pigments [14]. Now, several genes have been identified in black and yellow pigment synthesis [2], but the detail molecular mechanisms of different pigment synthesis were not well understood. In the present study, to better understand fish skin color genetics, iTRAQ was used to examine the differentially expressed proteins between the melanin synthesis pathway and pteridine synthesis pathway in common carp.

4.1. Skin Proteome Analysis

As a first step, 8064 protein kits were detected in common carp skin. GO analysis showed that most proteins were enriched in the immune system process, including endocytosis, phagosome, necroptosis, lysosome, and salmonella infection. These results confirmed that the major function of fish skin is to act as the first barrier of the immune system. It can provide protection against physical damage and assisted with the maintenance of homoeostasis by minimizing exchange between the animal and the environment. The goblet cells in the skin epidermis are responsible for the production of the mucosal layer [15]. The skin mucus is a composite of defensive molecules, including antibodies, together with factors of both the innate and acquired immune system [16]. The mucins are the major macromolecular components of mucus [17]. Mucins are heavily glycosylated proteins, which impart viscoelastic and rheological properties to mucosal layers [18]. In addition, we also found that many proteins are grouped into transporter activity, binding, and membrane-enclosed lumen in common carp skin proteomes. These results indicate that the skin in common carp also plays an important role in transporting gases, ions, nitrogenous waste products, and nutrients [19].

4.2. Up-Regulated Proteins in Black Skin Indicated the Molecular Mechanism of the Melanin Synthesis Pathway

Melanin is mainly synthesized by tyrosine within the melanosome [20,21]. In mammals and birds, two types of melanin are produced, the black or brown eumelanin and the lighter pheomelanin, but only eumelanin has been observed in teleost [22]. Eumelanin is synthesized by tyrosine within the melanosome of melanophores. This requires members of the tyrosinase family (TYP, DCT, and TYRPI) and probably Silver (SILV) [23–26]. Three melanosomal transporters (OCA2, AIMI, and SLC24A5) are crucial for proper melanin synthesis [27]. In the present study, we also found that several up-regulated proteins (Rad23a, mreg, tyrp1, and PMEL) in black skin were grouped into the melanogenesis pathway, including melanoregulin, melanocyte protein, and the UV excision repair protein, which might be responsible for the melanogenesis in the black skin of Jinbian carp. Given that the zebrafish golded mutant caused by slc24a5 deficiency is characterized by delayed and reduced development of melanin pigmentation, the up-regulated SLC25A4, SLC25A5, and SLC25A6 in the black skin may also associate with skin color variation [27,28]. However, we did not detect the different expression of TYP, DCT, and SILV between yellow and black skin, which indicated that these proteins might also play a role in the synthesis of yellow pigment. In addition, consistent with our recent transcriptome analysis, we also discovered that ACTC1, MYH6, and MYH7, involved in adrenergic signaling pathway, and ATP2A1, ATP2A2, ATP5F1, ATP5J, and COX5A, involved in oxidative metabolism pathway, were also up-regulated in black skin compared to yellow skin in proteome analysis [29]. Consequently, these genes could be candidate genes for the formation of yellow or black colors in Jinbian carp.
4.3. Up-Regulated Proteins in Yellow Skin Indicated the Molecular Mechanism of the Pteridine Synthesis Pathways

The yellow and reddish pteridine pigments are synthesized from GTP through the pteridine synthesis pathway in xanthophores. Three component pathways are involved in pteridine synthesis. Firstly, the GTP are converted into the tetrahydrobiopterin (H4biopterin), which is a cofactor for neurotransmitter synthesis and tyrosinase activity in melanophores. The second component is the regeneration pathway of oxidized H4biopterin. The third pathway shares several steps with the first one and leads to the formation of the yellow pigments, sepiapterin, and its derivatives [2,14]. During these processes, several genes were involved in the yellow pigmentation, such as the GTP cyclohydrolase I (GchI) [14], 6-pyruvoyltetrahydropterin synthase (Pts), sepiapterin reductase (Spr), xanthine oxidase/xanthine dehydrogenase (Xod/Xdh), and protein associated with Myc (PAM) [30]. In the present study, KEGG analysis showed that up-regulated proteins in yellow skin were preferentially grouped into several metabolism process, such as arginine and proline metabolism, pentose phosphate pathway, glycolysis/gluconeogenesis, fructose and mannose metabolism, carbon metabolism, and galactose metabolism. In addition, a few of the up-regulated proteins in yellow skin were also found to be related to nucleotide metabolism, such as GTPase IMAP family member 5 (GIMAP5), AMP deaminase 1 (AMPD1), adenosylhomocysteinase b (ahcy-b), and pyruvate kinase (PKM). These proteins may also play an important role in the yellow pigmentation.

In summary, we conducted a proteomic analysis among the yellow and black skin of Jinbian carp and the black skin of the wild common carp by using iTRAQ technology, and the results indicated that several up-regulated DEPs in black skin, including Rad23a, mreg, tyrp1, and PMEL, and several up-regulated DEPs in yellow skin, including GIMAP5, AMPD1, ahcy-b, and PKM, might be involved in the color variation in Jinbian carp.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-1729/10/10/226/s1, Table S1: Raw data analysis of the proteomic data among yellow skin in Jinbian carp (Y), black skin in Jinbian carp (B), and black skin in wild common carp (W).

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