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The metabolic adaptation of bile acids and cholesterol after biliary atresia in lamprey via transcriptome-based analysis

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ABSTRACT

Lamprey underwent biliary atresia (BA) at its metamorphosis stage. In contrast to patients with BA who develop progressive disease, lamprey can grow and develop normally, suggesting that lamprey has several adaptations for BA. Here we show that adaptive changes in bile acid and cholesterol metabolism are produced after lamprey BA. Among 1102 differentially expressed genes (DGEs) after BA in lamprey, many are enriched in gene ontology (GO) terms and pathways related to steroid metabolism. We find that among the DGEs related to bile acids and cholesterol metabolism, the expression of cytochrome P450 family 7 subfamily A member 1 (CYP7A1), sodium-dependent taurine cotransport polypeptide (NTCP) are significantly downregulated, whereas nuclear receptor farnesoid X receptor (FXR), multidrug resistance-associated protein 3 (MRP3), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), sterol O-acyltransferase 1 (SOAT1), and ATP binding cassette subfamily A member 1 (ABCA1) are remarkably upregulated. The changes in expression level are also validated by RT-qPCR. Furthermore, the level of high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) in juvenile serum is higher compared to larvae. Taken together, the findings collectively indicate that after BA, lamprey may maintain bile acids and cholesterol homeostasis in liver tissue by inhibiting bile acids synthesis and uptake, promoting its efflux back to circulation, and enhancing cholesterol esterification for storage as lipid droplets and its egress to form nascent HDL (nHDL). Understanding the possible molecular mechanisms of lamprey metabolic adaptation sheds new light on the understanding of the development and treatment of diseases caused by abnormal bile acid and cholesterol metabolism in humans.

1. Introduction

Lampreys belong to the Vertebrata, Cyclostomata, Petromyzoniformes, and Petromyzonidae. They are the living descendants of the

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world's most ancient jawless vertebrate, which lived 358 million years ago [1]. The life cycle of lamprey is complex, ranging from larva, metamorphosis, juvenile, and adult stages [2]. During metamorphosis, the intra- and extrahepatic bile ducts undergo degeneration and are ultimately lost, and the gallbladder disappeared [3–5]. This morphological change is similar to BA in humans.

The biliary system is important for maintaining the homeostasis of bile acids and cholesterol in the liver tissue. Bile acids are synthesized in the liver and secreted into the intestinal lumen through bile duct. 95% of bile acids were efficiently reabsorbed in small intestine and circulated back to the liver, only about 5% of bile acids were lost in the feces. This process was called enterohepatic circulation of bile acids. Cholesterol is the essential precursor for bile acids. The conversion of cholesterol into bile acids and its drainage as bile components through canaliculi into bile ducts are the main pathways for cholesterol excretion [6,7]. The disappearance of the biliary system after lamprey BA inevitably affects the metabolism of bile acids and cholesterol in the liver.

At normal physiological concentrations, bile acids play an important role in lipid digestion and absorption and act as signaling molecules to regulate body metabolism [8,9]. However, excessive bile acids in the liver cause oxidative stress and mitochondrial damage, which leads to the release of proinflammatory cytokines, that trigger an inflammatory response [10]. As for cholesterol, it is an essential component of mammalian cell membranes, regulating fluidity, permeability, and microstructures [11]. However, excessive cholesterol accumulation in the liver can lead to the development of Non-alcoholic steatohepatitis (NASH). Free cholesterol accumulates in Kupffer cells and hepatic stellate cells will trigger inflammation and fibrosis respectively. Furthermore, free cholesterol is highly toxic to organelles and will lead to ER stress and mitochondrial injury, which in turn lead to hepatocyte apoptosis. Therefore, the disruption of bile acids and cholesterol metabolism can lead to pathological consequences. However, in contrast to patients with BA who develop progressive disease, lamprey was able to grow and develop normally without inflammation and fibrosis [12,13], indicating that lamprey underwent adaptive changes in bile acid and cholesterol metabolism in order to adapt to BA during the evolutionary process. Regarding the adaptive changes in bile acid metabolism, it has been reported that to avoid the large bile acid accumulation in the liver, lamprey decrease the expression of CYP7A1, the rate-limiting enzyme for bile acid synthesis, to reduce the level of bile acid synthesis during the transformation from larval to juvenile stage [14]. Migratory adults likewise decrease hepatic synthesis of bile acids, but at the same time, increase bile acid efflux back to circulation and excrete excess bile acids from the body in the form of urine through the kidneys [15]. Upon sexual maturation, male lamprey synthesize a large amount of bile acid in the liver to produce bile acids pheromones, which are released through the gills via bloodstream and act as mating signal to recruit female lamprey to spawn [16–18]. However, until now, there is no related report about cholesterol metabolism adaptation after lamprey BA.

In this study, to further investigate the molecular mechanisms underlying the adaptive changes in bile acid and cholesterol metabolism following BA in lamprey, We first performed functional annotation of differentially expressed genes (DEGs) before and after BA in lamprey by using our own constructed local functional annotation package. Next, we analyzed and validated the expression changes of genes related to bile acid and cholesterol metabolism, and compared the changes in serum HDL and LDL levels. To the end, we revealed for the first time that after BA, lamprey may maintain bile acids and cholesterol homeostasis in liver tissue by inhibiting bile acids synthesis and uptake, promoting its efflux back to circulation, and enhancing cholesterol esterification for storage as lipid droplets and its egress to form nascent HDL (nHDL).

2. Materials and methods

2.1. Experimental animals

Lamprey (*Lampetra japonica*) larval (15 cm, 2–4 g) and juvenile stages (70 cm, 200–400 g), before and after BA respectively, including males and females, come from laboratory artificial fertilization and breeding. Every thirty lampreys were maintained in 200-L tanks of recirculating water at 4–6 °C. Lampreys handling and all of the experimental procedures were approved by the Animal Welfare and Research Ethics Committee of Liaoning Normal University and Dalian Medical University (Permit Number: SYXK2004-0029).

2.2. Local annotation data package construction

First, the *Petromyzon marinus* genome database was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/search/all/?term=aefg01). Based on the gene sequence information of the transcriptional expression matrix obtained in the article [19], gene sequences from genome database were extracted with a Python script. Next, the coding sequences (CDS) and corresponding amino acid sequences were predicted using TransDecoder (https://github.com/TransDecoder/TransDecoder/releases) with default parameters. Lamprey protein sequences were then functionally annotated by kofam_scan-1.3.0 (E value $<1e^{-5}$) using the KOfam eukaryotic database [20]. The gene ontology annotation was carried out by performing a local BLAST search against UniProt database (http://www.uniprot.org/) (E value $<1e^{-5}$). The AnnotationForge package (https://bioconductor.org/packages/release/bioc/html/AnnotationForge.html) is then used to create the local annotation data package for the enrichment analysis.

2.3. Functional enrichment analysis of DEGs between larval and juvenile stage

Gene expression matrix file was downloaded from the supplementary data of the article [19]. DEGs were screened out with the criteria of $\log2FC > 1$, $P < 0.05$. Functional enrichment analysis of DEGs was performed using ClusterProfiler program [21,22] against a local annotation data package. The diagrams were visualized using website a service (https://www.bioinformatics.com.cn/).

2.4. Construction of phylogenetic trees and analysis of conserved domains and protein tertiary structure

The homologous protein sequences of key genes for bile acids and cholesterol metabolism, cytochrome P450 family 7 subfamily A member 1 (CYP7A1), sodium-dependent taurine cotransport polypeptide (NTCP), multidrug resistance-associated protein 3 (MRP3), farnesoid X receptor (FXR), sterol O-acyltransferase 1 (SOAT1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and ATP binding cassette subfamily A member 1 (ABCA1) were obtained from the NCBI protein database (https://www.ncbi.nlm.nih.gov/). The accession numbers of these genes and the abbreviations of selected species used to construct the phylogenetic trees were listed in Supplementary Tables 1 and 2 respectively. The phylogenetic tree of the selected gene was constructed by Neighbor-joining method and Maximum likelihood through MEGA7 software (https://www.megasoftware.net/). Protein-conserved domains and tertiary structure were predicted by SMART (http://smart.embl-heidelberg.de/) and SWISS-MODEL (https://swissmodel.expasy.org/) respectively. Human protein tertiary structure was obtained from PDB database (https://www.rcsb.org). Their PDB ID are 3SN5, 7WSI, 6UY0, 3FLI, 8DJK, 6L47 and 7TBW respectively. The selected protein tertiary structure was superposed with human orthologs by SuperPose Version 1.0 (http://superpose.wishartlab.com/) online service and visualized by VMD software (https://www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD).

2.5. Lamprey primary hepatocytes isolation

Liver tissues were digested with collagenase (0.15% in DMEM, Thermo Fisher Scientific, Beijing, China) at room temperature for 15 min. After centrifugation at 3000 rpm for 10 min, cells were collected and cultured in DMEM medium supplemented with 1% penicillin/streptomycin (TransGen Biotech, Beijing, China) and 2% fetal bovine serum (TransGen Biotech, Beijing, China).

2.6. Quantitative reverse transcription PCR (RT-qPCR)

Total RNA was isolated using TRIzol (Sangon, Shanghai, China), and converted to cDNA by HiScript III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, Jiangsu, China). RT-qPCR was performed by using ChamQ® Universal SYBR qPCR Master Mix (Vazyme, Nanjing, Jiangsu, China). Primer sequences were described in Supplementary Table 3. Amplification was carried out with an initial step at 95 °C for 30 s, followed by 40 cycles of amplification (95 °C for 10 s, 60 °C for 30 s) by using a CFX96 qPCR system (Bio-Rad, Shanghai, China). GAPDH was used as an internal control. All results were representative of at least three independent experiments.

2.7. HDL-C and LDL-C assays

We used anesthetic MS-222 (meilunbio, Dalian, Liaoning, China) to anesthetize the lamprey. The blood was then collected from lamprey by cutting along the cloaca and centrifuged at 3000 rpm for 5 min. After centrifuging, the supernatant (serum) of blood from larval and juvenile stages was collected and HDL-C and LDL-C concentrations were measured using the HDL-C (A112-1) and LDL-C kit (A113-1-1) from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the manufacturer's instructions.

2.8. Statistical analysis

The student's t-test was performed to determine statistical differences between groups using the GraphPad Prism 7.0. $p < 0.05$ was

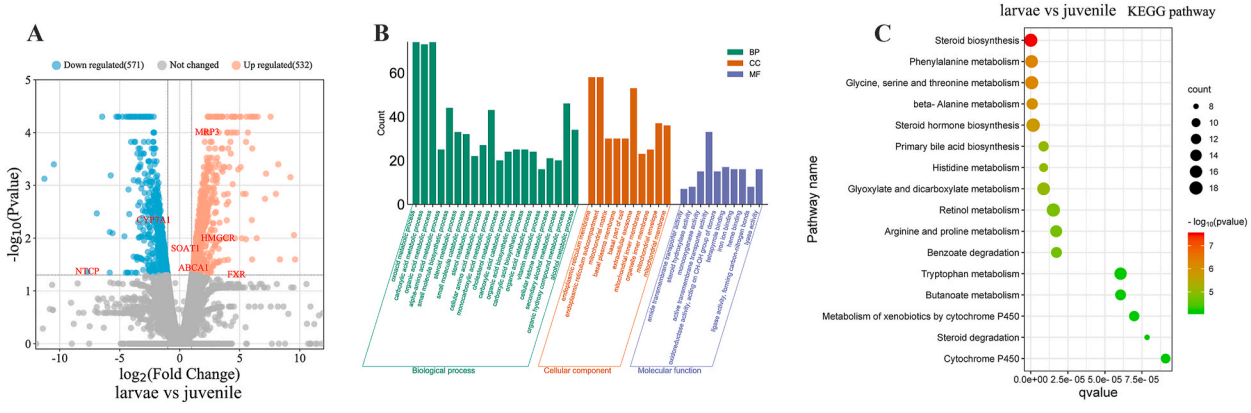

Fig. 1. Acquisition and functional annotation of DEGs. (A) The volcano plot of the DEGs between larval and juvenile stages. (B) BP, CC, and MF enrichment analysis of DEGs. The x-axis represents the annotation items of GO-BP, GO-CC, and GO-MF. The y-axis shows the gene counts of each term. (C) KEGG pathway enrichment analysis of DEGs. The x-axis shows the q_value of each term. The y-axis represents the annotation items of KEGG. Bubbles' sizes represent the number of genes associated with each term. The color of each bubble represents the adjusted p_value.An adjusted $p < 0.05$ was identified as significantly changed in GOs and KEGG analysis.

considered statistically significant. *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001 compared with the larva stage. All data were presented as mean ± S.E. of at least three independent experiments.

3. Results

3.1. Acquisition and functional annotation of DEGs

According to the expression matrix provided in the article [19], DEGs between juvenile (Jv) and larva (Lar) were screened out with the criteria of log2FC > 1 and P < 0.05. Eventually, 1102 DEGs were obtained (Supplementary File 1). Among them, 531 genes were up-regulated and 571 genes were down-regulated. The volcano plot of DEGs was shown in Fig. 1A. To understand the metabolic adaptation of bile acid and cholesterol after BA in lamprey, we performed functional annotation of 1102 DEGs by using our local annotation data package.

After the GO enrichment analysis, we altogether obtained 103 biological processes (BP), 16 cellular components (CC), and 27 molecular functions (MF). Notably, among the 103 biological processes, 22 items were associated with the synthesis, transport, storage, and metabolism of lipids, accounting for 21%. More importantly, according to the p_value ranking, we found that two of the top ten were associated with steroid metabolism (Fig. 1B). The top ten items and genes in BP, CC, and MF according to p-value were shown in Supplementary File 2.

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis was carried out to find out the signal pathway involved by DEGs. Altogether we obtained 93 KEGG pathways. Among them, three of the top ten ranked according to p-value were associated with steroid metabolism (Fig. 1C). They were steroid biosynthesis, steroid hormone biosynthesis, and primary bile acid biosynthesis. In particular, steroid biosynthesis ranked first in all pathways, which was worthy of our attention. The top ten items and genes ranked by KEGG according to p-value were shown in Supplementary File 3.

Based on these results, we found that steroid-related items were exhibited in both GO and KEGG enrichment, indicating that adaptive changes in bile acid and cholesterol metabolism occurred after lamprey BA.

3.2. Analysis of gene expression changes related to bile acid and cholesterol metabolism

To further understand the molecular mechanism of metabolic adaption of bile acids and cholesterol after lamprey BA, we analyzed the expression levels of genes related to bile acid and cholesterol metabolism using the expression matrix of different developmental

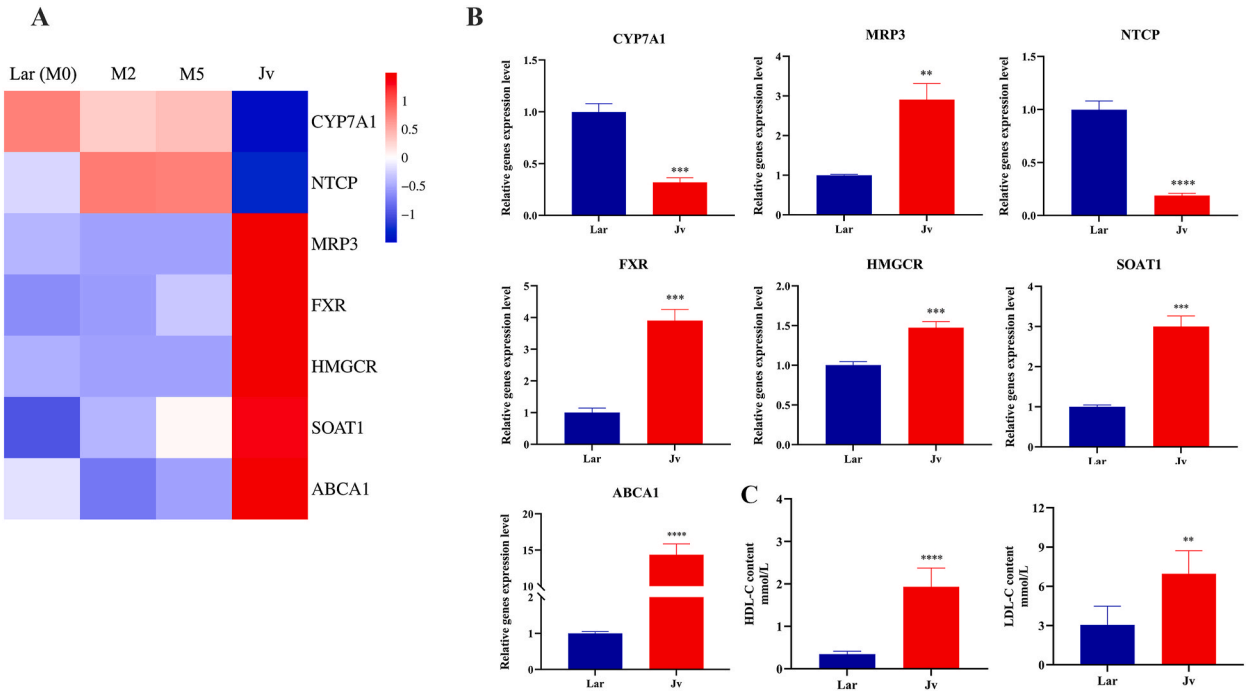

Fig. 2. Analysis of gene expression changes related to bile acid and cholesterol metabolism. (A)The heat map showed the changes in the expression of genes related to bile acid and cholesterol metabolism. (B) The relative mRNA expression levels of CYP7A1, MRP3, NTCP, FXR, HMGCR, SOAT1, and ABCA1 in lamprey hepatocytes at larval (Lar) and juvenile (Jv) stage were detected by RT-qPCR. (C) Serum HDL-C and LDL-C contents in larval and juvenile stages.Data shown are as mean ± S.E. of at least three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 versus larval stage.

stages in lamprey and created a heat map (Fig. 2A, Supplementary Table 4). The developmental stages, as defined by Youson and Sidon [3], include the larval stage (M0, before BA), the metamorphic stage (M2, gall bladder began to degenerate; M5, bile ducts were almost always obliterated), and the juvenile stage (Jv, after BA). As shown in Fig. 2A, we found that among the genes related to bile acid metabolism, CYP7A1 and NTCP were significantly down-regulated in the juvenile stage compared to M0, M2, and M5, while MRP3 and nuclear receptors FXR were significantly up-regulated. Among the genes related to cholesterol metabolism, those significantly

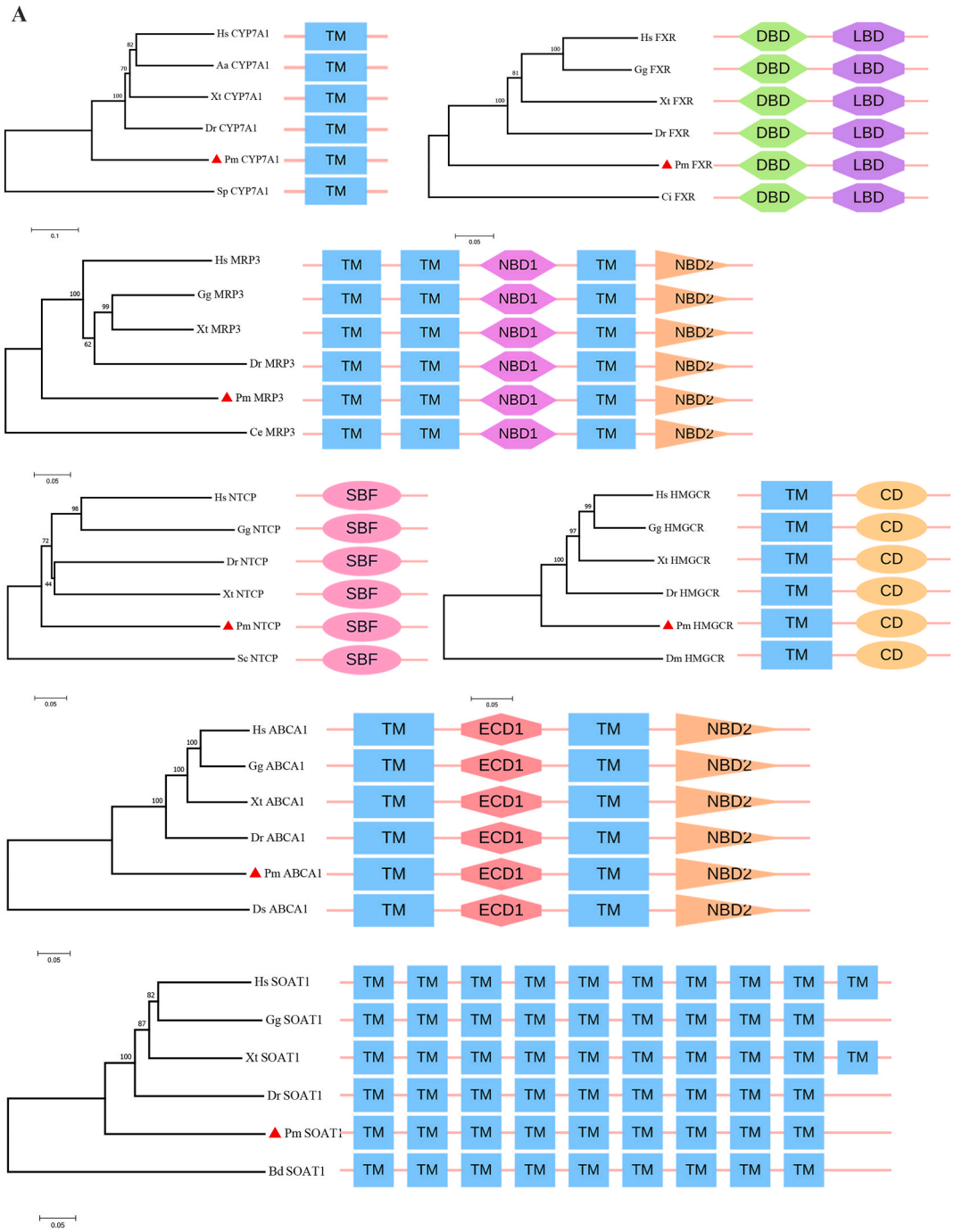

Fig. 3. Bioinformatic analysis of genes related to bile acid and cholesterol metabolism. (A) The phylogenetic tree and functional domains of CYP7A1, MRP3, NTCP, FXR, HMGCR, SOAT1, and ABCA1. (B) The overlap of predicted tertiary structures of human and sea lamprey proteins. Blue represents lamprey proteins. Red represents human proteins.
AbbreviationsTM, transmembrane domain; DBD, DNA binding domain; LBD, ligand binding domain; NBD, nucleotide-binding domains; CD, catalytic domain; ECD, extra-cellular domain; SBF, sodium bile acid symporter family.

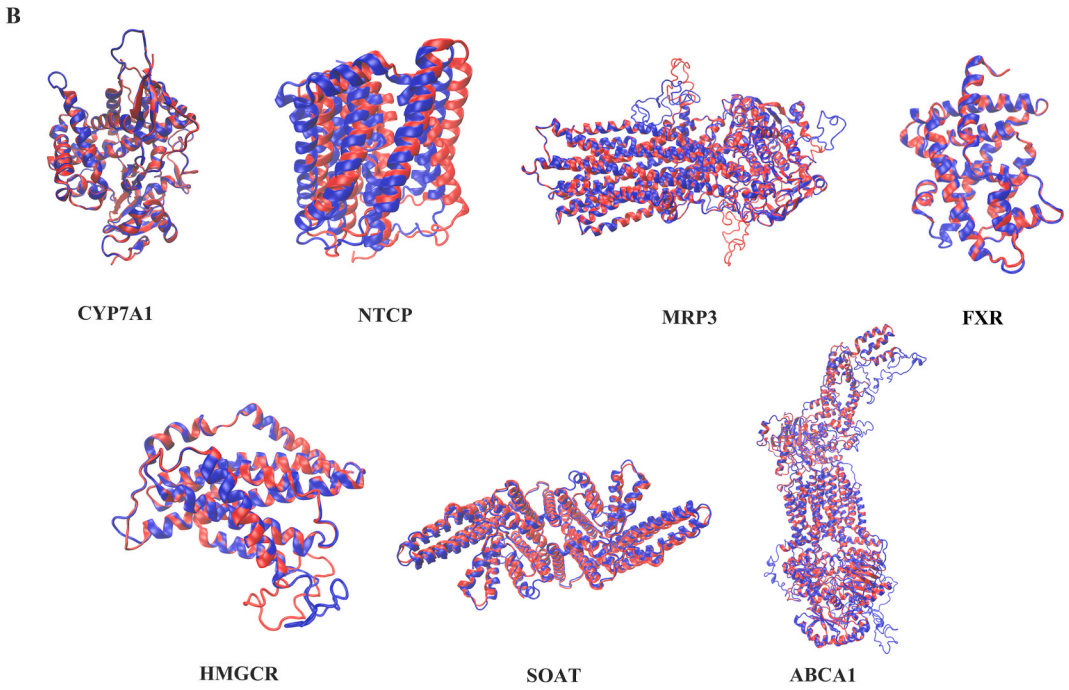

Fig. 3. (*continued*).

upregulated included HMGCR, SOAT1, and ABCA1. Next, RT-qPCR was performed to validate the above results. As shown in Fig. 2B, the experimental data were consistent with RNA sequencing data. Last, we compared the levels of HDL-C and LDL-C between juvenile and larva stages. As shown in Fig. 2C, the levels of HDL-C and LDL-C were significantly higher in Jv than in M0 stage.

3.3. Evolutionary conservation analysis of bile acid and cholesterol metabolism genes in lamprey

To confirm whether key genes for bile acid and cholesterol metabolism are evolutionarily conserved in lamprey, we first constructed NJ phylogenetic trees of CYP7A1, NTCP, MRP3, FXR, HMGCR, SOAT1, and ABCA1. As shown in Fig. 3A, all lamprey genes are located in vertebrate outgroups, suggesting that they may be the ancestral gene in vertebrates. Then, the domains of lamprey and its homologs were analyzed, and the results showed that the domains of these seven genes were highly conserved across species. Furthermore, when the tertiary structure of lamprey genes (predicted by homology modeling) was superposed with the corresponding human orthologs (Fig. 3B), the value of root mean square deviation (RMSD) was all below 3 (Table 1). indicating that these seven genes of lamprey have similar structures with the corresponding orthologs in human and may serve a similar function as in higher vertebrates.

4. Discussion

Metabolism is the basis for organism survival and reproduction. In order to adapt to changes in environment, habits, or animal characteristics, animal metabolism must also undergo adaptive changes. For example, metabolite adaption for extreme environments (e.g. plateaus, caves, etc.), for periodic changes in the environment (hibernation), and for food conversion and specialization (giant pandas adapt to a special diet of bamboo) et al. [23]. BA occurs in lamprey during metamorphosis. The biliary system plays an important role in regulating the metabolism of bile acid and cholesterol. The metabolic adaptive mechanisms of lamprey adapting to the loss of the biliary system to maintain bile acid and cholesterol homeostasis have not been reported.

We constructed a local enrichment analysis database and carried out the functional annotation of the DEGs between juvenile and larval stages. The results showed that large amounts of DEGs were enriched in the GO term like steroid metabolic process, sterol metabolic process, and cholesterol metabolic process. Many steroid-related KEGG pathways, such as steroid biosynthesis, steroid hormone biosynthesis, and primary bile acid biosynthesis, have a top p_value ranking. It suggested that the metabolic adaptation of

Table 1
The root mean square deviation (RMSD) of each protein.

Gene symbol	CYP7A1	NTCP	MRP3	FXR	HMGCR	SOAT1	ABCA1
RMSD	1.77	2.04	1.81	0.78	1.75	0.388	2.48

bile acids and cholesterol occurred after BA in lamprey.

Regarding the adaptive changes in bile acid metabolism after BA in the lamprey, it has been reported that the lamprey avoids the large bile acid accumulation caused by BA by decreasing the expression of CYP7A1, the rate-limiting enzyme for bile acid synthesis, changing the composition of bile acids, and increasing bile acid efflux into the bloodstream, where they are further excreted through the kidneys or gills [14,15]. It has been documented migratory adults excreted excess bile acids from the body in the form of urine through the kidneys [15]. Upon sexual maturation, male lamprey release bile acids pheromones, which act as mating signal to recruit female lamprey to spawn, through the gills via bloodstream [16–18]. In the present study, through data mining and experiments, we found that not only CYP7A1 was downregulated, but also the NTCP gene that promotes bile acid absorption was significantly downregulated. In contrast, the expression of MRP3 that promote the efflux of bile acid back to the circulation was remarkably upregulated, which hinted to us that lamprey may not only reduce bile acid synthesis but also reduce bile acid absorption and promote bile acid excretion to the systemic circulation to maintain the homeostasis of bile acids metabolism (Fig. 4). And bile acids that enter the bloodstream, as described in the literature, are excreted out of the body through the kidney and gills. Our results also showed a significant upregulation of FXR expression. FXR is a member of the nuclear receptor family. FXR plays a central role in the regulation of bile acid metabolism homeostasis [24,25]. It was reported that FXR induced the expression of small heterodimer partner (SHP). SHP in turn acts as co-repressor to inhibit the transactivating activity of hepatocyte nuclear factor 4 alpha (HNF-4α) and liver receptor homolog 1 (LRH-1), leading to the inhibition of CYP7A1 gene transcription [26,27]. Similarly, transcription factors, HNF-4α, and retinoid X receptor alpha (RXRα)-retinoic acid receptor alpha (RARα) complex, regulating NTCP expression were also repressed by SHP [28]. However, after blasting against NCBI or our local lamprey genome database, we found that SHP orthologs did not exist in lamprey genome. Then how FXR regulates the expression of CYP7A1 and NTCP needs us to launch a search in the future.

The conversion of cholesterol into bile acids and its drainage as bile components through canaliculi into bile ducts are the main pathways for cholesterol excretion [6,7]. The decrease of bile acid synthesis and the loss of the biliary system in lamprey after BA will inevitably lead to obstruction of cholesterol excretion. At the same time, we found that the expression level of HMGCR, the rate-limiting enzyme for cholesterol synthesis, was significantly increased after BA in lamprey, which means that endogenous synthesis of cholesterol was increased. Then, under the situation that cholesterol excretion was blocked, while the endogenous synthesis of cholesterol was increased, how lamprey maintains intrahepatic cholesterol homeostasis to avoid excess cholesterol accumulation in the liver? In the liver, excess cholesterol can be esterified by SOAT1 to form cholesteryl esters and stored in the form of cytosolic lipid droplets [29]. Cholesterol can also be transported, either passively or actively, to apolipoprotein A1 (apoA1) to form HDL under the mediation of ABCA1 [30,31]. We speculated that the inability of biliary cholesterol excretion would affect the other pathways for cholesterol metabolism in the liver. Consistent with our speculation, our results showed that the expression level of SOAT1 and ABCA1 and the level of serum HDL-C and LDL-C were all remarkably increased. The upregulation of SOAT1 expression will lead to the enhancement of lipid droplets. Our previous work showed that a large number of lipid droplets were indeed present in lamprey liver hepatocytes at the juvenile stage [32]. ABCA1 is the major transporter that facilitates the efflux of cholesterol to poorly lipidated apoA1 to form nHDL [33]. Moreover, the level of the nHDL contributes 80% of the serum HDL-C content [34], indicating that the expression level of ABCA1 determines the level of HDL-C. In the present study, the level of LDL-C was also increased after BA, which is consistent with the report that ABCA1 is also involved in regulating LDL-C levels, as not only HDL-C levels but LDL-C levels were decreased after the ABCA1 gene was knockdown [35,36]. These combined results demonstrated that after lamprey BA, an adaptive change of cholesterol metabolism in the liver occurred (Fig. 4). First, lamprey may promote the esterification of free cholesterol (FC) by

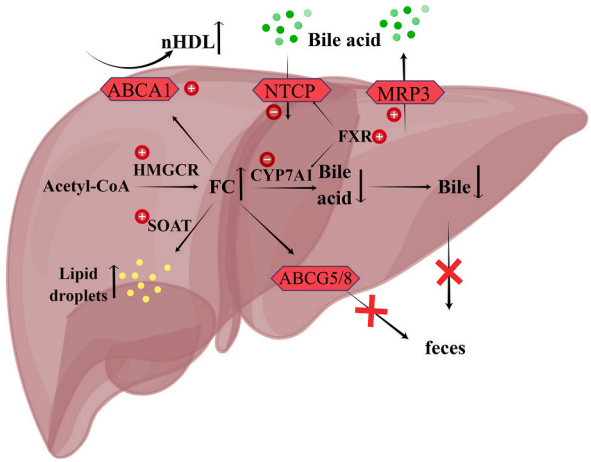

Fig. 4. Schematic diagram illustrating the adaptive changes in bile acids and cholesterol metabolism in lamprey liver after BA.
After lamprey BA, CYP7A1, and NTCP were down-regulated, and MRP3 and FXR were significantly up-regulated, which indicated that lamprey maintains bile acids homeostasis by reducing the synthesis and absorption of bile acids, enhancing bile acids efflux back to circulation. Lamprey maintains cholesterol homeostasis by increasing the biogenesis of lipid droplets and nascent HDL through increasing SOAT1 and ABCA1 gene expression, respectively.

increasing the expression level of SOAT1, thus storing excess cholesterol in the form of lipid droplets to avoid lipotoxicity from excess intrahepatic cholesterol caused by the absence of the biliary system. Second, the upregulation expression of ABCA1 in lamprey after BA promotes the excretion of intrahepatic cholesterol efflux into systemic circulation in the form of nHDL, thus providing materials for the generation of steroid hormones for steroidogenic organs. However, there are literature showed that at lamprey sexual maturation stage, lamprey gonadal tissue only produces minute amount of sex hormones [37], but the liver needs to synthesize large amounts of bile acids, which yield the sex pheromones PZS and 3kPZS [16–18,38]. Therefore, we speculated that cholesterol stored in lipid droplets, as well as cholesterol in HDL that is returned to the liver via receptor, provide the raw material reserves for the synthesis of bile acid sex pheromones by the liver during sexual maturation.

After BA, the lamprey undergoes metabolic adaptations in both bile acid and cholesterol metabolism. In future work, further exploration of the molecular mechanisms underlying the regulation of expression of key genes in bile acid and cholesterol metabolism after BA may provide new insights for understanding the treatment of diseases associated with abnormal bile acid and cholesterol metabolism in humans.

Author contribution statement

Qipeng Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Jilong Pan, Yingying Zhu, Jindi Liu, Jiarui Li, Pengju Han: Analyzed and interpreted the data.
Yue Pang, Meng Gou, Jun Li, Peng Su: Contributed reagents, materials, analysis tools or data.
Qingwei Li: Conceived and designed the experiments.
Yan Chi: Conceived and designed the experiments; Wrote the paper.

5. Data availability statement

Data will be made available on request.

6. Declaration of interest's statement

The authors declare no known conflict of interest.

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Ethics approval

The animal experiments were performed in accordance with the regulations of the Animal Welfare and Research Ethics Committee of the Institute of Dalian Medical University's Animal Care protocol (Permit Number: SCXK2008-0002).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All authors make sure that all data and materials support published claims and comply with field standards.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e19107.

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