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ORIGINAL ARTICLE

Characterization of Embryo Transcriptome of Gynogenetic Olive Flounder *Paralichthys olivaceus*

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Abstract Olive flounder *Paralichthys olivaceus* is an important commercially cultured marine flatfish in China, Korea, and Japan. Gynogenesis, via meiogynogenesis and mitogynogenesis, shows advantages in breeding and sex control, but the low survival rate, especially for mitogynogenesis, limits its application. In this study, we sequenced the embryo transcriptomes of gynogenetic haploid, meiogynogenetic diploid, mitogynogenetic diploid, and common diploid flounder and investigated their respective genetic characteristics by analyzing differentiated expressed genes. Compared with common diploid, the gynogenetic haploid showed significant downregulation in notch signaling and wingless-related integration site (Wnt) signaling pathways, which may be the source of haploid syndrome. In both meiogynogenesis and mitogynogenesis, several upregulated genes including complement C3, formin-2, and intelectin may be related to increased survival compared to the haploid. The downregulation of immune system and energy metabolism-related genes caused retarded development of gynogenetic diploids compared with the common diploid. These data provided new and important information for application of artificially induced gynogenesis to aquaculture.

Keywords Olive flounder *Paralichthys olivaceus* · Transcriptome · Meiogynogenesis · Mitogynogenesis · Haploid · Differentiated expressed genes

Introduction

Gynogenesis refers to a form of all-female reproduction mainly in lower vertebrates, such as some reptiles, amphibians, and teleosts (Neaves and Baumann 2011). Natural gynogenesis can complete meiosis stimulated by heterologous sperm (Zhang et al. 2015b). In gynogenetic zygote, male pronucleus does not fuse with the female pronucleus, and development is primarily controlled by maternally derived genes. Thus, gynogenesis could be used to produce isogenic lines or clones that are useful in breeding and sex-controlled studies and has been successfully applied in fish. In addition, gynogenesis provides important material for construction of genetic map, location of quantitative trait locus, and study of sex determination mechanism (Mei and Gui 2015). Artificially induced gynogenesis has long been achieved in fish (Makino and Ozima 1943; Purdom 1969; Cherfas 1975; Nagy et al. 1978). Artificial gynogenetic haploid can be induced by activation of eggs with UV-irradiated spermatozoa, but the resulting haploid is non-viable and dies before or soon after hatching, a condition known as haploid syndrome (Arai 2001; Zhong et al. 2009). Viable gynogenetic diploid can be obtained by diploidization of the haploid maternal chromosome set by inhibiting either the second polar body extrusion or the early cleavage (Streisinger et al. 1981; Zhu et al. 2006). The former is called meiogynogenesis, and the latter is called mitogynogenesis. Meiogynogenesis develops from a female

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with assumed heterozygous gene locus; thus, the loci on distal areas of chromosomes are considered heterozygous because of gene-centromere recombination, while those on proximal areas are homozygous (Thorgaard et al. 1983). Homozygous lines cannot be produced by meiogynogenesis, but it is hypothetically possible to produce isogenic lines by two, three, or more cycles of meiogynogenesis. Mitogynogenesis is homozygous for all loci and could potentially be used to produce cloned fish via second cycle gynogenesis. With its genetic uniformity, mitogynogenesis allows estimation of genetic correlations and detection of genotype/environment interactions and phenotypic plasticity for complex traits such as sex and gonad differentiation, stress response, and disease resistance. However, mitogynogenesis produces higher deformity and lower survival rates than does meiogynogenesis, due to direct effects of the treatment and deleterious recessive mutations, as well as asynchronous embryo development (Arai 2001; You et al. 2008; Wang et al. 2011). The small numbers of progeny produced by mitogynogenesis surviving to maturity display dramatically reduced fertility, low fecundity, and poor egg quality (Suzuki et al. 1985; Komen and Thorgaard 2007). The source of these phenomena is poorly understood, and the use of mitogynogenesis in aquaculture is limited. Artificially induced meiogynogenesis has been carried out in more than 100 fish species, while mitogynogenesis has been artificially induced in few (Komen and Thorgaard 2007). Previous studies on gynogenetic fish focused on genetic analysis, physiological and morphological quantitative traits, biological characteristics, and aquacultural performances (Del Valle et al. 1994, 1996; Yamamoto 1999; Arai 2001; Komen and Thorgaard 2007). The mechanism of haploid syndrome remains unknown at the molecular level. The low viability of gynogenetic fish has been taken as evidence for the absence of differential parental imprinting: male- and female-specific epigenetic methylation of certain genes during gametogenesis (Corley-Smith et al. 1996).

Over the past decade, significant progress has been made in development and application of transcriptome sequencing, which can show comprehensive gene expression patterns and differential expression profiles. By analyzing the gonad transcriptome, Liu et al. (2015) revealed the molecular effect of depletion of primordial germ cells of gynogenetic fish on sex differentiation. Reports of the embryonic transcriptome of gynogenetic fish, which can provide new information on aspects of meiogynogenesis and mitogynogenesis, are scarce.

Olive flounder *Paralichthys olivaceus* is an economically important flatfish in China, Japan, and Korea, providing abundant high quality protein and unsaturated fatty acids. Increased demand has resulted in rapid reduction of stock and the development of artificial breeding and culture. Flounder reach sexual maturity at 3 to 4 years, limiting the efficiency of traditional selective breeding. Gynogenesis is a potential alternative (Yamamoto 1999; You et al. 2001). Meiogynogenesis can rapidly produce offspring with desirable characteristics through increased genetic similarity, and mitogynogenesis is an effective means of obtaining homozygous clones. As in other fish, the application of mitogynogenesis in flounder is limited by asynchronous embryo development, low survival rate and sterility (You et al. 2001, 2008; Zhang et al. 2015a). Gonad transriptome analyses have been performed to identify differentially expressed genes that may cause reproductive dysfunction in mitogynogenetic flounder (Zhang et al. 2015a). The molecular mechanism of gynogenetic asynchronous embryo development remains to be elucidated.

In this study, we sequenced the embryo transcriptomes of gynogenetic haploid, meiogynogenetic diploid, and mitogynogenetic diploid together with a common diploid flounder and identified differentially expressed genes. These data provide a genomic resource for future study of fish gynogenesis and contribute to elucidating haploid syndrome. Characteristics of gynogenetic diploid can be demonstrated from gene expression profiles to provide important information applicable to artificially induced gynogenesis.

Materials and Methods

Induction of Gynogenesis and Sample Collection

The induction of gynogenesis in olive flounder *P. olivaceus* was conducted at the Shenghang Fish Farm, Weihai. Meiogynogenic diploid and mitogynogenetic diploid were produced as previously reported (Wang et al. 2008; You et al. 2008), and common diploid and haploid were simultaneously performed. Briefly, the semen from one male was diluted 50 times with Ringer's solution at 0–2 °C (which does not activate sperm motility) and immediately irradiated under ultraviolet light of wavelength, 254 nm, at 36,000 ergs/mm^2. Eggs were stripped from one female, and approximately 25 % were fertilized with untreated sperm for the common diploid. Remaining eggs were fertilized with the irradiated sperm and divided into three aliquots for the haploid control and induction of mitogynogenesis and meiogynogenesis. Meiogynogenesis was induced by cold-shock at 0–2 °C for 45 min. Mitogynogenesis was induced by hydrostatic pressure treatment at 60 MPa for 6 min at 85 min after fertilization. All fertilized eggs were incubated in nets floating in a 16-m^3 pool under conditions of *L*/*D* 14:10, temperature 15.0 ± 0.2 °C, and >6 mg/L oxygen saturation. Gynogenesis was confirmed by visual observation of embryo development (You et al. 2008). Approximately 1500 embryos from each group were collected at ~70 h post-fertilization and instantly frozen in liquid nitrogen for RNA extraction. The experiment was conducted in

triplicate, each replicate using sperm and eggs from a different fish.

RNA Isolation and cDNA Library Construction

Total RNA was isolated from each sample using TRIzol reagent (Omega, USA). The RNA concentration and A260/A280 ratio were measured using the NanoDrop™ 2000 (Thermo Scientific, Canada). RNA integrity was evaluated using the 2100 Bioanalyzer (Agilent, USA), with the RNA 6000 Nano kit. The mRNA was enriched by Oligo (dT) magnetic beads (Invitrogen, USA) and disrupted into short fragments by adding fragmentation buffer (NEBNext Ultra RNA Library Prep Kit for Illumina). The first-strand cDNA was synthesized using random hexamers, and the second-strand cDNA was synthesized using DNA polymerase I (NEBNext Ultra RNA Library Prep Kit for Illumina) based on the first-strand cDNA. The synthesized cDNA was enriched by Ampure beads and trimmed with poly(A) and adaptor. These purified cDNA fragments were used to construct the cDNA library with the TruSeqTM DNA sample Prep kit-set (NEBNext Ultra RNA Library Prep Kit for Illumina). The quality, including the concentration and insert size, of the cDNA library was ascertained by Qubit2.0, Agilent 2100, and qPCR. Finally, 12 cDNA libraries were sequenced on Illumina 2500 platform with 100 bp paired-end strategy at the Beijing BioMarker Technologies Company.

Illumina Sequencing, Function Annotation, and Classification

The raw sequence data were trimmed by filtering out adaptor and low-quality reads with *Q* scores lower than 30. The clean reads from the biological replicate libraries of each group were jointly de novo assembled into unigenes by Trinity software (Grabherr et al. 2011). The quality of the unigene libraries was ascertained by tests of mRNA fragment randomness, insert size, and sequencing saturation. All unigenes were searched against databases of NCBI non-redundant (NR) proteins, Cluster of Orthologous Groups (COG), protein family (Pfam), gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) using BLAST software (cutoff *E* value 1E-5; HMMER cutoff *E* value 1E-10) (Altschul et al. 1997). The protein coding sequences of the unigenes were further determined by TransDecoder software.

Differential Expression Analysis

Fragments per kilobase of transcript per million mapped reads (FPKM) were calculated to standardize the gene expression level (Trapnell et al. 2010). The repeatability among biological replicates was evaluated by Pearson's correlation coefficient (r) (Schulze et al. 2012). The Benjamini-Hochberg method was used to determine the threshold of false discovery rate (FDR) (corrected p value) in multiple testing. An FDR <0.05 and fold change ≥ 1 were used to identify differentially expressed genes (DEGs) between groups (common diploid, haploid, meiogynogenetic diploid, and mitogynogenetic diploid) through DEGseq (edgeR) (Anders and Huber 2010). All DEGs between groups were subjected to GO, COG, and KEGG annotation and KEGG enrichment analysis. In addition, we identified DEGs among the biological replicates to present within-group differences.

Results

Assembly and Annotation

The Illumina RNA-Seq output comprised 12 libraries with a range of 10 to 12 million reads after quality trimming and filtering. In all libraries, the proportion of qualified Q30 reads was greater than 86.88 %. All clean read data have been deposited in the NCBI SRA database under accession number SRP075889. The filtered reads were assembled to produce 71,816 unigenes of mean size 1089 bp. The majority of unigenes were between 200 and 500 bp in length. Homology searches against the NR database annotated 38,990 unigenes of which 19,768 were greater than 1 Kb in length. Through GO analysis, 27,323 unigenes were assigned by molecular function (35,790), cellular components (72,913), and biological processes (89,023). The assigned GO terms comprised *binding* and *catalytic activity* (molecular function), *cell* and *cell part* (cellular component), and *cellular process* and *metabolic process* (biological process) (Fig. 1). Through COG analysis, 26,474 unigenes were annotated, mainly distributed in general function prediction, replication, recombination and repair, transcription, signal transduction mechanisms, translation, ribosomal structure and biogenesis, substance (amino acid, carbohydrate, inorganic, lipid, secondary metabolites) transport, and metabolism (Fig. 2). Mapped to KEGG database, 16,113 unigenes were assigned KEGG orthology (KO) numbers and further mapped to 229 reference canonical pathways.

Comparative Gene Expression Profiles of Gynogenetic Haploid and Common Diploid

The Pearson correlation coefficient ($r > 0.94$) showed that the repeatability of replicates of haploid and common diploid was valid. Compared with the common diploid, 530 upregulated and 1128 downregulated unigenes were identified in the haploid. The clustering of DEGs between the haploid and common diploid was exhibited in Fig. S1. The annotated DEGs were listed in Table S1. The upregulated genes were

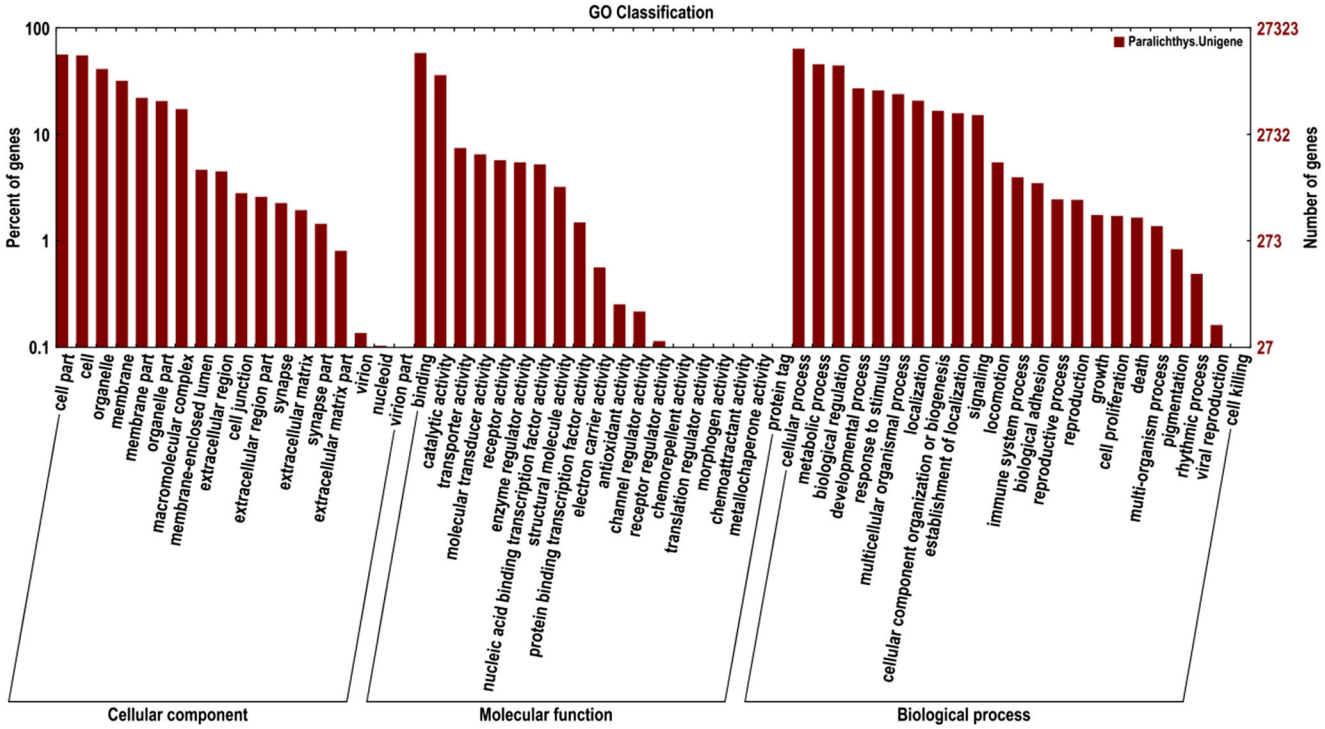

Fig. 1 Gene ontology (*GO*) assignment class. The *horizontal axis* represents the GO secondary term. The *right vertical axis* is the number of genes assigned to the GO term, and the *left vertical axis* gives the percent of genes assigned to the GO term

represented by complement C3, polymerase delta-interacting protein 3, H/ACA ribonucleoprotein complex subunit 1, sarcoplasmic/endoplasmic reticulum calcium ATPase 1, nebulin, and formin-2. The downregulated genes comprised the rho GTPase-activating protein 35, acyl-coenzyme A thioesterase 11, hormone sensitive lipase, ankyrin-3, phosphatidylinositide phosphatase SAC1-B, E3 ubiquitin-protein ligase, ceramide synthase 2, solute carrier family 23

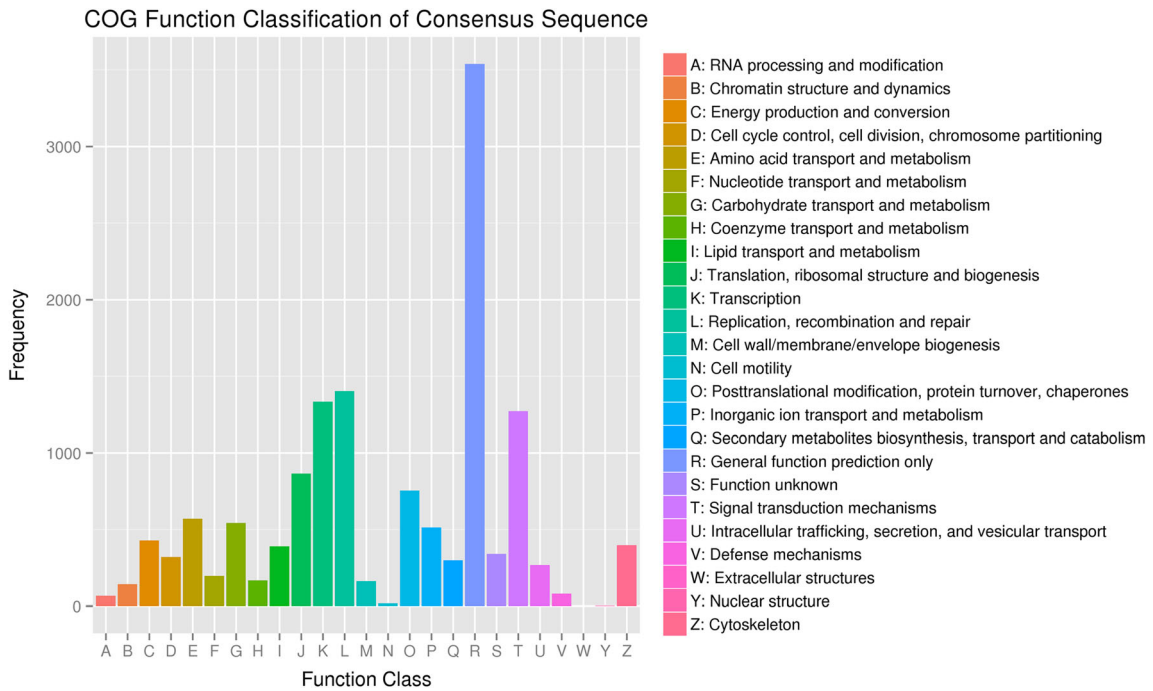

Fig. 2 Cluster of orthologous groups of proteins (*COG*) assignment class. The *horizontal axis* represents the COG term. The *vertical axis* represents the frequency of genes assigned to the COG term

member 2, and amyloid beta A4 protein. The GO analysis showed DEGs to be widely distributed in GO terms (Fig. S2). The COG analysis showed that DEGs were mainly associated with signal transduction mechanisms, replication, recombination and repair, transcription, translation, ribosomal structure and biogenesis, substance transport, and metabolism (Fig. S3). KEGG statistical analysis showed DEGs were related to focal adhesion, regulation of actin cytoskeleton, the notch signaling pathway, the wingless-related integration site (Wnt) signaling pathway, and the mitogen-activated protein kinases (MAPK) signaling pathway (Fig. S4). KEGG enrichment showed the notch signaling pathway to be the most prominent of the enriched pathways (Fig. S5). Eight downregulated genes, including neurogenic locus notch homolog protein 1, delta-like proteins A and B, protein jagged-2, disintegrin and metalloproteinase domain-containing protein 17, and CREB-binding protein and one upregulated gene, C-terminal-binding protein 2, make up this pathway (Fig. 3). Frizzled receptor genes (fzd1, fzd3, fzd5, fzd8), receptor-related protein genes (lrp5, lrp6), and the dvl3 gene of the Wnt signaling pathway showed a downregulated pattern in the haploid (Fig. 4).

Comparison of Expression Profiles of Gynogenetic Diploids and Haploid

The Pearson correlation coefficient ($r > 0.8$) showed that the repeatability of three replicates produced by meiogynogenesis and mitogynogenesis was suitable for DEGs analysis. Compared with the haploid, 32 upregulated and 49 downregulated unigenes were identified in the mitogynogenetic diploid (Table S2). The upregulated genes included complement C3, formin-2, intelectin, laminin subunit beta-1, transcription factor COE2, and eukaryotic translation initiation factor 4. The downregulated genes included fructose-bisphosphate aldolase C, apolipoprotein D, microfibril-associated glycoprotein 4, and phosphoglycerate kinase 1. KEGG enrichment showed that these DEGs were involved in glycolysis, pentose phosphate pathway, fructose, and mannose metabolism (Fig. S6). Compared with the haploid, 33 upregulated and 31 downregulated unigenes were identified in the meiogynogenetic diploid (Table S2). The upregulated genes comprised in formin-2, intelectin, complement C3, serpina1 protein, and titin. The downregulated genes included fructose-bisphosphate aldolase C, interferon-stimulated gene 15, butyrophilin subfamily 1 member A1, and 6-phosphofructokinase type C. KEGG enrichment showed that these DEGs were involved in the pentose phosphate pathway, fructose and mannose metabolism, glycolysis, and galactose metabolism (Fig. S7).

Comparison of Expression Profiles of Gynogenetic Diploids and Common Diploid

Zygotic allelic genes of the common diploid were derived from both the paternal and maternal genomes, while the allelic genes of gynogenetic diploids were from the

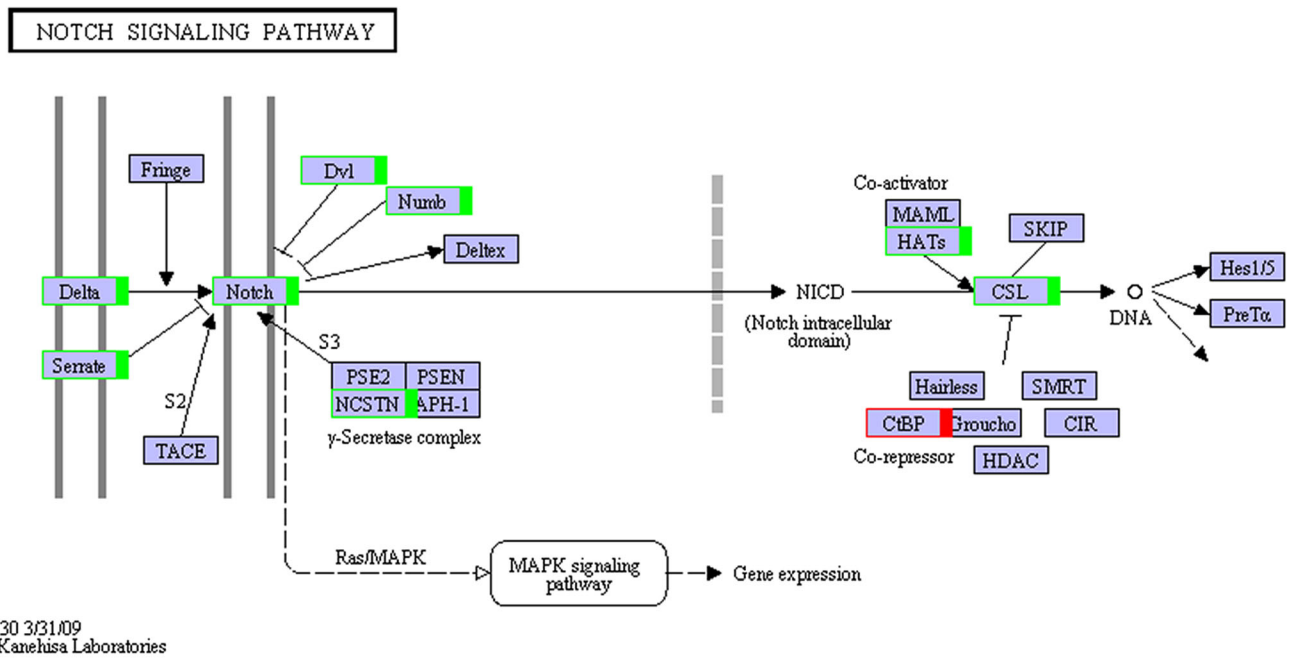

Fig. 3 Notch signaling pathway. *Blue background* indicates genes identified in the flounder transcriptome. *Red border* indicates upregulated genes in haploid. *Green background* indicates downregulated genes in haploid

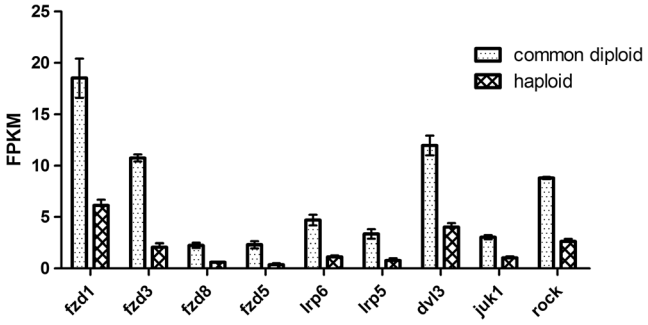

Fig. 4 Downregulated genes associated with the Wnt signaling pathway. *Horizontal axis* represents genes. *Vertical axis* indicates fragments per kilobase of transcript per million mapped reads (*FPKM*)

maternal genome only. Relative to the common diploid, 30 downregulated unigenes were identified in the mitogynogenetic diploid, while only 3 downregulated unigenes were identified in the meiogynogenetic diploid (Table S3). None upregulated gene was identified in gynogenetic diploids. In mitogynogenesis, the downregulated unigenes were chiefly related to general function prediction, carbohydrate transport and metabolism, and inorganic ion transport and metabolism and included guanine nucleotide-binding protein G, fructose-bisphosphate aldolase C-B, phosphosulfate synthase 2, beta-crystallin B1, and crystallin gamma M2b. KEGG enrichment revealed DEGs to be involved in the pentose phosphate pathway, fructose and mannose metabolism, glycolysis, sulfur metabolism, and selennocompound metabolism (Fig. S8). In meiogynogenesis, only eosinophil peroxidase was annotated as downregulated gene.

Comparative Expression Profiles of Meiogynogenetic and Mitogynogenetic Diploid

No differentially expressed gene was identified when comparing meiogynogenetic and mitogynogenetic diploid.

The Within-Group DEGs Among Replicates

We further investigated within-group DEGs among biological replicates (Fig. 5). There is almost no difference within-group DEGs among biological replicates of common diploid. There are also fewer differences within-group DEGs among replicates of haploid. However, there are larger numbers of within-group DEGs to be identified among biological replicates of mitogynogenesis or meiogynogenesis. The common diploid showed the fewest number of DEGs among biological replicates. Both mitogynogenesis and meiogynogenesis showed more within-group DEGs than the common diploid.

Discussion

Using second-generation sequencing technology, we sequenced the transcriptomes of gynogenetic flounder embryos. Transcriptome comparison of gynogenetic haploid and common diploid contributed to understanding the molecular mechanism for haploid syndrome. Meiogynogenesis and mitogynogenesis restore the diploid genome by different mechanisms. It is still unclear how diploidization changes the transcriptome profile. Although meiogynogenetic and mitogynogenetic diploid of flounder are viable, similar to other fish, gynogenesis especially mitogynogenesis results in low survival rate and delayed development (You et al. 2001, 2008). This transcriptome analyses contributed to illumination of novel characteristics of gynogenetic diploids.

Transcriptome Characteristics of Gynogenetic Haploid

Gynogenetic haploid was produced by activation of eggs with UV-irradiated spermatozoa. The alterations in genetic expression due to lack of the paternal allelic genes were considerable. Several DEGs were enriched in vital signal transduction pathways. The notch signaling pathway regulates many aspects of metazoan development and tissue renewal (Artavanis-Tsakonas et al. 1999). The mis-regulation or loss of notch signaling underlies a wide range of human disorders, from developmental syndromes to adult-onset diseases and cancer (Yuan et al. 2014). Downregulation of notch receptors and ligands may result in development abnormalities (Artavanis-Tsakonas et al. 1999). Thus, haploid syndrome may be associated with the mis-regulation of the notch signaling pathway.

The Wnt signal transduction pathway was also shown to be downregulated in the haploid. Wnt signaling is involved in virtually every aspect of embryonic development and controls homeostatic self-renewal in adult tissues (Clevers 2006;

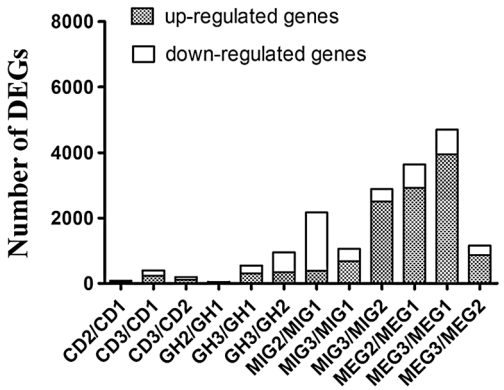

Fig. 5 The number of within-group DEGs among replicates. The *horizontal axis* represents the comparison among three replicates in each group. The *vertical axis* is the number of within-group DEGs. *CD* = common diploid, *GH* = gynogenetic haploid, *MIG* = mitogynogenesis, *MEG* = meiogynogenesis

MacDonald et al. 2009). The Wnt pathway is activated by the Wnt ligand binding to the transmembrane frizzled receptor (Fzd). Canonical Wnt pathway activation requires low-density lipoprotein receptor-related protein 6 (LRP6) or LRP5 as coreceptor (Clevers 2006; MacDonald et al. 2009). Although Wnt protein showed no difference in expression between the haploid and common diploid, Wnt signals could be strongly affected by downregulation of Fdz and coreceptor genes. In addition, the downregulation of Dvl3 influences Fz-Dvl interaction and Wnt-induced LRP6 phosphorylation. This points to the downregulation of Wnt receptor genes as the vital factor interfering with embryonic development in haploid.

Upregulated Genes May Contribute to the Survival of the Gynogenetic Diploids

The gynogenetic diploid undergoes diploidization of the maternal chromosome set by inhibiting either the second polar body extrusion or the early cleavage. We observed few genes differentially expressed in meiogynogenesis and mitogynogenesis. This result is contrary to what had been expected from a gene dosage effect between common diploid and gynogenetic haploid. Several upregulated genes, including complement C3, formin-2, and intelectin, were identified in both meiogynogenetic and mitogynogenetic diploids. Complement C3 plays a central role in the activation of the complement system. Processing by C3 convertase is the primary reaction in both classical and alternative complement pathways (Sfyroera et al. 2015). Formin-2 plays a role in response to DNA damage, cellular stress, and hypoxia by protecting CDKN1A against degradation and, hence, acts in stress-induced cell cycle arrest (Belin et al. 2015). Formin-2 also acts as an actin-binding protein that is involved in actin cytoskeleton assembly and reorganization, contributing to cytoskeleton dynamics and intracellular transport (Leader et al. 2002). Intelectin is active in the innate immune system as a receptor for bacterial arabinogalactans (Tsuji et al. 2001) and for lactoferrin (Suzuki et al. 2001). These upregulated genes may be related to embryo survival in the gynogenetic diploids.

Several downregulated genes are related to glycolysis, cell adhesion, and the immune system. The relationship between these downregulated genes and embryo survival needs further study. The KEGG enrichment for DEGs was found to be similar in meiogynogenesis and mitogynogenensis compared to haploid. Meiogynogenetic diploid consistently showed no DEGs with the mitogynogenentic. Mitogynogenensis resulted in a lower survival rate than meiogynogenesis, which cannot be explained by a difference in gene expression profiles. The embryos used in this study survived near to hatching, which may be the reason for similar transcriptome profiles. The embryo transcriptome at early stages of development may explain the difference in survival rates and is a topic for further study.

Downregulated Genes Cause Delayed Development of Gynogenetic Diploids

As we observed, both meiogynogenesis and mitogynogenesis could delay development at the embryo stage (You et al. 2001, 2008). In this study, we identified several genes in meiogynogenesis and mitogynogenesis that were downregulated compared to the common diploid. In mitogynogenesis, fucolectin-1 was primary among downregulated genes. Fucolectin-1 can recognize blood group fucosylated oligosaccharides including A, B, H, and Lewis B-type antigens and acts as defensive agent just reported in Japanese eel *Anguilla japonica* (Honda et al. 2000). The glycolytic process may be influenced by downregulated expression of fructose-bisphosphate aldolase C-B, which is involved in step 4 of the sub-pathway that synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose. Beta- and gamma-crystallin are the dominant structural components of the vertebrate eye lens. They are expressed during development of the normal eye and linked to vascular remodeling (Graw 1997; Weadick and Chang 2009). In meiogynogenesis, eosinophil peroxidase was the only annotated downregulated gene. Human eosinophil peroxidase mediates tyrosine nitration of secondary granule proteins in mature resting eosinophils and shows significant inhibitory activity of *Mycobacterium tuberculosis* H37Rv by inducing bacterial fragmentation and lysis (Borelli et al. 2003; Ulrich et al. 2008). These downregulated genes are associated with the immune system and energy metabolism, which may be the reason for delayed development of the gynogenetic diploids.

The downregulated genes in the gynogenetic diploids may be the candidate imprinting genes from the paternal genome. Imprinting is commonly found in mammals (Kono et al. 2004). Imprinting leads to unequal expression of maternal and paternal alleles in the embryo. Some imprinted genes exhibit a bias for expression in either the maternal or paternal allele, rather than complete silencing (Khatib 2007). In the present study, the replacement of the paternal genome by the maternal genome may be the source of the downregulation of the imprinting genes. The perturbation of the balance of maternally and paternally expressed imprinted genes may lead to instability of the transcriptome, as reflected by considerable transcriptome difference among biological replicates of the gynogenetic diploids. The instability of the transcriptome may be the source of the low survival rate in gynogenetic diploids. Further study to demonstrate the existence of imprinting genes and their role in embryonic development is warranted.

Conclusion

Gynogenesis shows advantages in breeding and sex control, but the low survival rate, especially for mitogynogenesis, limits its application. In this study, we sequenced the pre-hatching embryo transcriptomes of haploid, meiogynogenesis, mitogynogenesis, and common diploid. The downregulation of the notch signaling pathway and Wnt signaling pathway may be the reason for haploid syndrome. Several upregulated genes, including complement C3, formin-2, and intelectin, may be associated with embryo survival of gynogenetic diploids. The downregulated genes of the immune system and energy metabolism may be related to delayed development of gynogenetic diploids. These data provide new understanding of artificially induced gynogenesis in flounder.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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