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Quantitative proteomic reveals the dynamic of protein profile during final oocyte maturation in zebrafish

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ABSTRACT

The molecular mechanisms underlying final oocyte maturation in zebrafish (*Danio rerio*) remain poorly understood. The present study aimed to employ iTRAQ approach for a comprehensive characterization of during zebrafish oocyte maturation proteome and for comparison between fully-grow immature and mature oocytes prior to ovulation. A total of 1568 proteins were identified, which was representing the largest zebrafish isolated oocytes proteome dataset to date. Differential expression analysis revealed 190 proteins significantly changes between immature and mature oocytes, which 136 proteins were up-regulated and 54 proteins were down-regulated in mature oocytes comparison with immature oocytes. Functional analysis revealed that these differential proteins were mostly involved in cellular response to estrogen stimulus, cellular components, extracellular region, and enzyme regulator activity, etc. The revealed differentially changes in protein expression patterns associated with oocyte maturation suggest that several of the examined proteins, such as vitellogenin(Vtg3), protein S100(S100A10), 17-beta hydroxysteroid dehydrogenase(HSD17B1), pentaxin, zona pellucida (ZP3.2), elongation factor1-alpha, caluemnin B, and 14-3-3 protein may play a specific role during zebrafish final oocyte maturation. These data will provide powerful information for understanding the molecular mechanism underlying zebrafish oocyte maturation, and these proteins may potentially act as markers to predict control oocyte maturation of zebrafish oocytes.

1. Introduction

Teleost oocytes undergo a series of regulated events during follicular development in the ovary, from primordial follicles to ovulation. At the end of follicular growth, fully-grown immature oocyte arrested at the prophase of meiosis I, attains competence to undergo meiotic G2-M1 transition. Subsequently undergoes final oocyte maturation which consists of the movement of the oocyte nucleus(germinal vesicle, GV) to a cortical position and germinal vesicle breakdown (GVBD) [1,2]. Final oocyte maturation occurs prior to ovulation and is a prerequisite for successful fertilization [1], whose impairments lead to low reproductive performance [3]. Several endocrine and/or local autocrine/porcine factors and potential interplay between apparently disparate intro-oocyte signaling events during oocyte maturation have been revealed of active research in teleost [1,2,4]. The gene expression profile of oocytes during maturation process and identify differential expression genes/transcripts in oocyte at different development stages using genomics and transcriptomics have been performed. Transcriptomic investigations of zebrafish oocytes during their maturation allowed an overview of the total mRNA composition of ovary [5]. Moreover, it was demonstrated maternal transcriptome composition of mature zebrafish oocytes seems to be tightly regulated with a distinct mother-specific signature [6]. However, merely having complete sequences of genomes is not sufficient to elucidate biological function, and there is rarely correlated well between genes and the protein complement [7,8].

Proteomics strategies have been applied in the research of teleost ovarian development, including goldfish [9], Eurasian perch [10], Atlantic herring [11], Persian sturgeon [12], zebrafish and gilthead sea bream [8,13,14]. In these studies, two-dimensional electrophoresis (2D-GE), multi-dimensional protein identification

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technology(MudPIT), and selected reaction monitoring(SRM) were employed to identify the proteome profile of the gonad differentiation, adult zebrafish ovary, and developing oocyte. However, the number of proteins identified is quite limited. A moderated number of 60 proteins were identified in fully grown zebrafish ovarian follicles using a gel-based approach and around 600 proteins were identified in zebrafish oocytes at different stages prior to maturation by combined 2D-GE and MudPIT [5,14]. Moreover, 1379 proteins were detected in adult zebrafish ovary by 2D-LC-MS/MS(false discovery rate(FDR) 3%) [8]. These results provide important information on the factors regulating the oocyte development in fish.

However, despite significant information obtained from zebrafish isolated follicle or ovary, a holistic proteome analysis of final oocyte maturation process in fish is limited. Particularly, little is known about the molecular pathways in final oocyte maturation. The use of isobaric tagging for relative and absolute quantization(iTRAQ) combined with LC-MS/MS approach has become more effective and more powerful methodology in proteomics analysis [15]. With this approach, zebrafish proteomes have been reported for gill [16], central nervous system [17], embryos [18], and liver [19]. The number of proteins identified with iTRAQ varies from more two hundred to over six thousand. With the current advances of proteomic technology, an extensive analysis of proteome dynamics during zebrafish final oocyte maturation would provide valuable insights for future oocyte related research in fish. Meanwhile, taking into account the growing interest in the zebrafish models and the great potential of oocyte maturation biomarker discovery, there is an urgent need to understand the basic oocyte protein profile. Therefore, the current study was aimed to investigate the protein expression profile of zebrafish final oocyte maturation, identify the differentially proteins in follicle-enclosed oocyte (hereinafter referred to as oocyte) at full-growth immature and mature stages using iTRAQ technology.

2. Materials and methods

2.1. Animals and oocyte collection

The WT sexually mature zebrafish was a Tübingen strain propagated in our lab according to the standard procedure. Fish was maintained at 28 °C ± 1 °C on a 14 h light cycle with lights on at 0800 h. Experimental protocols were approved by the Institutional Animal care and Use Committee at Shanghai Ocean University.

Gravid females and adult males were kept separately for at least 7days prior to their use in the experiment [20]. Briefly, one the day before sampling, fishes were released at the ratio of one female: two males in aquaria at 2000h. The next day five to six females were autopsied, and ovaries were removed into 60% Leibovitz-15 culture medium. The fully-grown immature oocyte change in cytoplasm from opaque to transparent due to GVBD as easily identifiable marker for final oocyte maturation [21]. Therefore, the fully-grown immature oocyte(GV) stage and mature oocyte(GVBD) stage was collected at 0500 h and 0700 h, respectively. Two parallel pools were prepared for each stage (~200oocytes/pool). Then, oocytes was washed three times with PBS buffer, snap-frozen in liquid nitrogen and stored at −80 °C until protein extraction.

2.2. Protein extraction and iTRAQ

After oocyte protein extraction, the samples were labeled according to the instructions of iTRAQ Reagent-8Plex, Mltiplex Kit (AB Sciex). Protein samples were labeled 113(GV1), 114(GV1), 115 (GVBD1) and 116(GVBD2) and then pooled and dried by centrifugal evaporation, provided in Supplementary Materials and Methods.

2.3. Peptides separation by high pH HPLC and LC-MS/MS analysis

The labeled peptides were separated by high-pH-reverse-phase LC column(C18, 5 μm 4.6 × 250 mm)(Waters). The peptides were eluted in Buffer A(98%H_2O, 2% acetointrile (ACN), pH10.0) and Buffer B(98% ACN, 2% H_2O, pH10.0) at a flow rate of 0.7 mL/min. Forty eluted fractions were collected and combined to make 6 fractions and reduce peptide complexity, according to protein properties.

The eluted fractions were lyophilised in a centrifugal speed vacuum concentrator and dissolved with 0.5% formic acid. The ultimate 3000 nano HPLC system coupled QExactive mass spectrometer (Themo) was utilized to iTRAQ analysis.

2.4. Data processing and protein identification

The MS/MS raw data were searched against the Uniprot database for *danio rerio*(uniprot_danio_rerio_170221. fasta) for peptide identification and quantification using Mascot 2.2 and Proteome Discoverer 2.0 (Thermo Fishiers). Proteins with FDR≤0.01 were qualified for further quantification data analysis. All identified proteins between immature and mature oocytes were compared to identify differences of proteins expression involved final oocyte maturation. Fold change cutoff ratio(FC) of ≥1.2 or ≤0.833 was set as the threshold to identify differently expressed proteins. A minimum of one unique peptide was required to identify and relatively quantify a protein.

2.5. Bioinformatics analysis

Functional classifications of differentially expressed proteins (DEPs) were performed using GO (http://www.geneontology.org). Enrichment analyses were realized using all the annotated proteins identified as a reference and using Uniprot accession IDs as input. The DEPs were assigned to KEGG database for pathway enrichment analysis. Clusters of Orthologous Groups of proteins (KOG) were employed.

2.6. Real-time RT-PCR

Total RNA from GV and GVBD oocytes samples were extracted separately using RNAiso Plus(Takara) according to the manufacturer's instruction, and 1 μg total RNA was used for reverse-transcription reaction using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara). The obtained cDNA was diluted to 1:10 with ddH_2O and used as a template for real-time RT-PCR using ef1a as an internal control. Specific primers for real-time PCR were listed in Supplementary Materials and Methods Table 2. The qRT-PCR amplifications were performed with a 20 μL reaction mixture using SYBR® Premix Ex Taq™ (QIGAN), carrying out on the ABI7500 Real-Time PCR Detection System (Applied Biosystems). The threshold cycle (Ct) value was analyzed with ABI Optical System Software, the $2^{-\Delta\Delta Ct}$ method to be used to determinate the expression level of each sample. Three replicates were carried out for each gene using immature and mature oocytes.

3. Results

3.1. The proteomes of zebrafish oocytes

Proteins were extracted from the pooled from 5 to 6 females and two biological replicate were be collected for each GV and GVBD oocytes stages, thus presenting average proteome patterns of immature and mature oocytes. A total of 1568 proteins were identified from the labeled samples, which were common in both

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