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Acute immune stress improves cell resistance to chemical poison damage in SP600125-induced polyploidy of fish cells *in vitro*

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ABSTRACT

Previous research has indicated that the small compound, SP600125, could induce polyploidy of fish cells, and has established a stable tetraploid cell line from diploid fish cells. In order to explore how fish cells maintain homeostasis under SP600125-stress *in vitro*, this study investigates impacts of SP600125-stress on intracellular pathways, as well as on regulation of the cellular homeostasis feedback in fish cells. Transcriptomes are obtained from the SP600125-treated cells. Compared with unigenes expressed in control group (crucial carp fin cells), a total of 2670 and 1846 unigenes are significantly upregulated and downregulated in these cells, respectively. Differentially expressed genes are found, which are involved in innate defense, inflammatory pathways and cell adhesion molecules-related pathways. The SP600125-stress enhances cell-mediated immunity, characterized by significantly increasing expression of multiple immune genes. These enhanced immune genes include the pro-inflammatory cytokines (IL-1β, TNF-α, IL-6R), the adaptor signal transducers (STAT, IκBα), and the integrins (α2β1, αMβ2). Furthermore, mitochondria are contributed to the cellular homeostasis regulation upon the SP600125-stress. The results show that acute inflammation is an adaptive and controlled response to the SP600125-stress, which is beneficial for alleviating toxicity by SP600125. They provide a potential way of breeding fish polyploidy induced by SP600125 in the future research.

1. Introduction

The chemical compound, SP600125, is a specific, reversible and cell-permeable ATP competitive inhibitor [1]. It was widely used in a variety of studies *in vitro* and *in vivo*, ranging from cancer [2–4] to immune diseases [5,6]. It has been reported that SP600125 prevents the c-Jun phosphorylation by inhibiting JNK function [1], and affects various cellular responses, ultimately disrupting normal cellular activity such as autophagy, inhibition of apoptosis and survival [7–9]. SP600125 might strongly inhibit cell proliferation in various cancer cells, including breast cancer [10], multiple myeloma [11], B-lymphoma [12], cervical cells [13] and megakaryocytic cell line [14]. As an anthrapyrazole compound, SP600125 was also used to treat cancer, autoimmune and neurodegenerative diseases [15–18].

Zhou et al. have found that SP600125 treatment can induce tetraploid cells from caudle fin cells of crucian carp (*carassius auratus) in vitro* such that a new polyploidization method was established [19]. By transplanting the nuclei of SP600125-induced tetraploid cells into unfertilized eggs of crucian carp, tetraploid fish were obtained [19]. Polyploidy has many advantages, such as rapid growth and disease resistance, over diploidy, and plays an important role in germplasm innovation and breeding new varieties [20]. However, polyploidization often occurs almost as a result of abnormal cell division, where the stability depends on the rapid restructure of the genome and changes in gene regulation [21]. Particularly, it is still unclear how fish cells could maintain homeostasis in the case that they are treated by SP600125 *in vitro*. In this study, by transcriptome analysis based on the Illumina sequencing technology, we intend to answer what are the cell responses to SP600125-stress, especially for controlling the cell death. Our results will lead to better understanding of the mechanism for maintaining stability or establishing cellular homeostasis under SP600125-stress.

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2. Materials and methods

2.1. Cell culture and SP600125 treatment

Crucian carp was maintained at the Ministry of Education National Center of Polyploidy Fish Breeding, Hunan Normal University. Animal experiments were conducted in strict accordance with the recommendations in the Guidelines for the Care and Use of Laboratory Animals of the National Advisory Committee for Laboratory Animal Research in China, and were approved by the Animal Care Committee of Hunan Normal University.

Fish were anesthetized with 100 mg/L MS-222 (Sigma, St. Louis, MO, USA) before dissection. Cells obtained from the caudle fin were cultured in Dulbecco's modified Eagles medium (DMEM; Sigma) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (FBS; Invitrogen), 0.1% 2-mercaptoethan (2-ME; Invitrogen), 1 mM sodium pyruvate (Invitrogen) and 1 mM nonessential amino acids (Invitrogen). Cells were grown in 5% CO_2 at 28 °C. For cryopreservation, 10% dimethyl sulfoxide (DMSO; Sigma) and 90% FBS were used.

For polyploid cells induction, 100 μm SP600125 ($C_{14}H_8N_2O$, Merck, Germany) was added to the cell culture medium described above when the tail fin cells reached 80–90% confluence. DMSO (10%) was used as the solvent for SP600125 [19,22].

2.2. Cyto-histological observation

For electron microscopy, cells were fixed in 2.5% glutaraldehyde for 2 h, and then treated with 1% OsO_4 for 2 h. After dehydration through an ethanol gradient, the specimens were embedded in Epon812 resin (TAAB, USA). The sections were double-stained with uranyl acetate and lead citrate, and were examined by an electron microscope (JEM-1230, JEOL, Tokyo, Japan).

2.3. Analysis of cellular DNA content and ATP content

Cells were digested into a single-cell solution by trypsin, and washed twice. After filtered through a 40 μm cell strainer, the cells were incubated with 2 μg/ml Hoechst 33342 (Invitrogen) and 50 μM Verapamil (Sigma) for about 15 min. Then, the cells were detected by flow-cytometer (Partec, German). Cellular ATP contents were assessed using an ATP Assay Kit (cat# A095-1, Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

2.4. Sampling of transcriptome data

All samples were stored in RNA later (Ambien Life technologies, Grand Island, NY, USA) at −80 °C. Total RNA was purified using Trizol (Invitrogen) and quantified with an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). The cDNA libraries were synthesized using mRNA fragments as templates with the TruSeq RNA sample preparation kit V2 (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. *De novo* assembly was carried out with Trinity using default parameters to generate unigenes. Then, we collected the mRNA-Seq data of cultured fin cells and SP600125-treated cells.

2.5. Analysis of differentially expressed transcripts

FPKM, fragments per kilobase of exon per million fragments, was used to calculate the transcript expression levels. The adjusted *P*-values were used for the false discovery rate (FDR) in multiple hypothesis testing, which uses the Benjamini-Hochberg correction to determine the difference significance between samples. Unigenes with FDR ≤ 0.001 and $|\log2Ratio| \geq 2$ were considered to be differentially expressed genes for further analysis. Gene ontology (GO) enrichment analysis was carried out with the Blast2 GO v2.5.0 software [23]. GO terms with FDR < 0.05 were considered to be significantly enriched.

2.6. Quantitative real-time PCR analysis

Total RNA was extracted from the crucian carp fin cells and the SP600125-treated cells. Each RNA sample was treated with PrimeScript™ RT Reagent Kit with DNA Eraser (Takara) to remove residual genomic DNA and reverse transcribe into cDNA. The quantitative real-time PCR (qRT-PCR) analysis was performed using the Prism 7500 Sequence Detection 140 System (Applied Biosystems) with a miScript SYBR Green PCR kit (Qiagen, Valencia, CA, USA). The primers for the qRT-PCR, which were designed by Primer Express® Software Primer 5.0, are shown in Table S1. The PCR was carried out on Stratagene Mx3000 P (Agilent). For each sample, the qRT-PCR analysis was performed on three biological replicates.

2.7. Statistical analysis

Statistical analysis was performed using the Student's *t*-test for comparison of two groups or one-way analysis of variance (ANOVA) for comparison of more than two groups, followed by Tukey's multiple comparison test. For multiple testing, a Bonferroni *post hoc* test of *P* values was performed. Statistical calculations were performed using Graph Pad Prism (Graph Pad, San Diego, CA, USA). Data were expressed as mean ± SEM of at least three independent experiments. '$P < 0.05$' was considered to be statistically significant.

3. Results

3.1. Observation of SP600125-treated cells

After treatment with 100 μM SP600125 for 48 h, there was some cell death (Fig. 1 a-b). Flow cytometry analysis also demonstrated that the number of cells significantly decreased after SP600125 treatment compared with the control (Fig. 1c–d). Furthermore, there were some obvious changes in the structure of SP600125-treated cells. For example, the number of mitochondria increased and their shape changed from short to long bars in SP600125-treated cells, compared with the control cells (Fig. 1 e-f). In addition, Fig. 1e–f showed that the intracellular bodies were greatly increasing and there was cell secretory hyperactivity.

3.2. Transcriptome changes in SP600125-treated cells

A comparative analysis of the transcriptome was carried out on cultured fin cells and cells treated with 100 μM SP600125 for 48 h. After *de novo* assembly, unigenes were obtained by the paired-end method of Trinity and TGICL clustering with a max length of 22511bp, min length of 300 bp, average length of 867.06bp and N50: 1267bp. To identity differentially expressed unigenes (DEGs) in the control cells and the SP600125-treated cells, the expression of assembled unigenes was measured by the FPKM method. We found that 2670 genes were upregulated and 1846 were downregulated in the SP600125-treated cells, compared with the control cells (Fig. 2a). Further GO analysis demonstrated that the majority of the transcripts were associated with "biological regulation", "cellular component", "cellular reproduction", "extracellular and cell adhesion", "hormone secretion", "immune system process" and "response to stimulus" (Fig. 2b).

To verify the reliability of the transcriptome data, 18 DEGs were analyzed by qRT-PCR, which included the 7 upregulated and 11 downregulated genes. As seen in Fig. 3, the fold-change values obtained by qRT-PCR were consistent with the values obtained by the RNA-seq for 17 ones among the selected genes, except for the AKT gene.

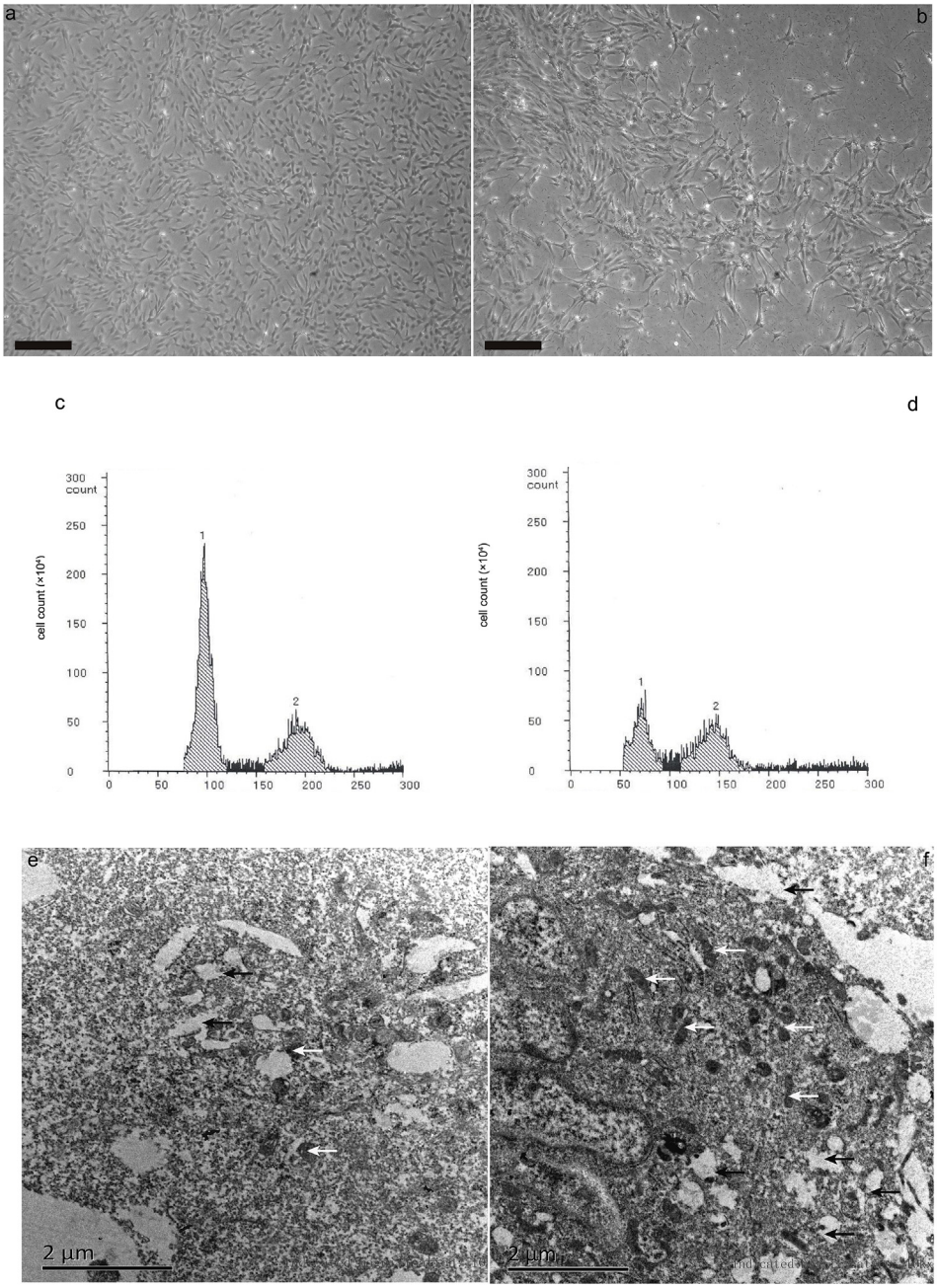

Fig. 1. SP600125-treated fish cells *in vitro*. (a) and (b) show the cultured cells from crucial carp fin (a), and the SP600125-treated cells in 48 h (b). The scale bar equals 100 μm. (c) and (d) show the DNA contents of the crucial carp fin cells (c), and those of the SP600125-treated cells (d) by flow cytometry. The horizontal axis indicates the fluorescence intensity, and the vertical axis indicates the cell volume. (e) and (f) show the ultrastructure of crucial carp fin cells (e) and that of the SP600125-treated cells (f). The white arrows point to mitochondria, and the black arrows point to the secretory vesicles. The scale bar equals 2 μm.

3.3. KEGG pathway analysis of DEGs

To elucidate the regulation mechanism of the SP600125-treated cells, significant DEGs were annotated by KEGG. A total of 794 differentially expressed genes were annotated into 18 immune-related pathways by the KEGG database. Some of these pathways were associated with ECM-receptor interaction (15.2%), Cell adhesion molecules (CAMs) (8.9%), Cytokine-cytokine receptor interaction (8.7%), Measles and Toxoplasmosis (6.8%), Leukocyte transendothelial migration (6.2%), Endocytosis (5.6%) and Toll-like receptor signaling pathway (5.5%) (Table S2).

3.4. Annotation of immune-relevant DEGs

Many important immunity elements were significantly upregulated at 48 h after SP600125 treatment. As shown in Table 1, they include the cytokines and cytokine receptors (IL-1β, TNF-α, IL-8, IL-11, CD40, IL-6R, IL-2R, DOCK2), the adaptor signal transducers (STAT1, IκBα, STAT3, SLIP, HSP70, MHC-II), the integrin's (α2β1, αMβ2, TSP, ITGB2, αVβ3), the chemokines and chemokine receptors (CXCR3, CXCR4, CXCR11), the T cell receptor (TCR) signaling molecules (CD8, CD9, CD40), and the apoptosis-related genes (CASP8).

The qRT-PCR analysis further validated that the mRNA transcripts of some immune-relevant genes, such as COX-2, IL-1β, STAT1, STAT3, HSP70 and CASP8, were notably increased in the SP600125-treated

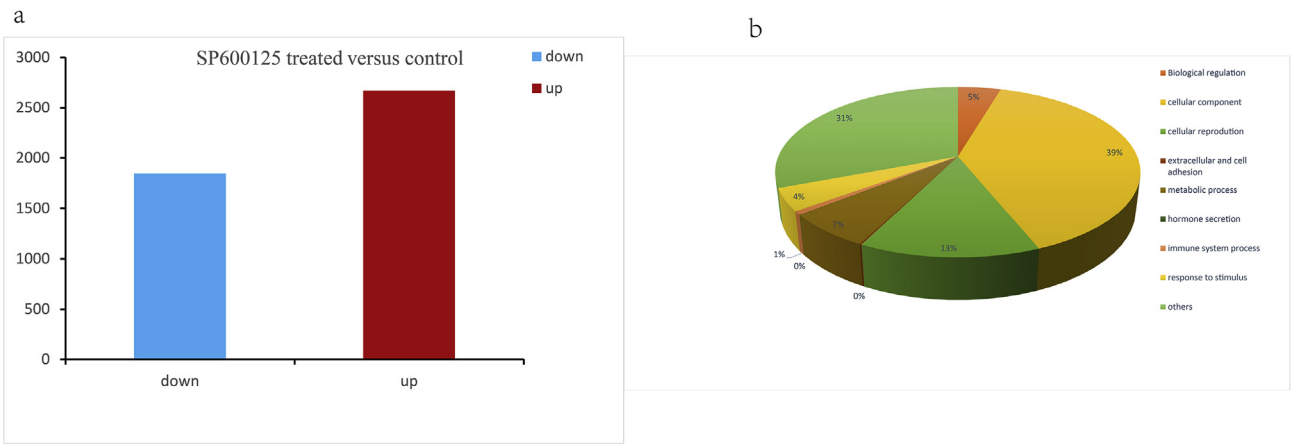

Fig. 2. Differently expressed genes (DEGs) and Gene ontology (GO) enrichment of the DEGs in the SP600125-treated cells and in the control ones of crucial carp. (a) Compared with the control group, there were 2670 upregulated genes and 1846 downregulated genes in the SP600125-treated cells. There were two expression levels: The upregulated expression is represented by the red color, while the blue color stands for the downregulated expression. (b) GO biological process categories enrichment of DEGs in the SP600125-treated and in the control cells of crucial carp. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

cells compared with the control (Fig. 4).

3.5. Analysis of mitochondria-related genes

Mitochondria play an important role in energy (ATP) production and act as a sensitive organelle susceptible to damage [24]. The results in Table 2 indicate that the gene expression levels of many important mitochondrial elements were significantly upregulated or downregulated after the SP600125 treatment, which included the mitochondrial import receptor subunits (TOM6), glucose transporters (GLUT1), the key glycolytic enzymes (PFKFB, ENO, LDH), the mitochondrial CMP kinase (CMPK), the dimethylglycine dehydrogenase (DMGDH), the cytochrome C oxides subunit (COX8, COX5B), and the electron transport chain genes (CoQ5).

Glucose, a primary energy source of the cell, undergoes cytosolic metabolism (glycolysis) and mitochondrial bioenergetics (the TCA cycle and respiratory chain) [25]. By SP600125 treatment, ATP content of the cells decreased (Fig. 5A). By qRT-PCR analysis, it was further demonstrated that the mRNA expression levels of GLUT1 and key glycolytic enzymes such as HK1, PFBFK, PGAM, ENO and LDH were significantly downregulated after the SP600125 treatment (Fig. 5B–a). However, as seen in Fig. 5B–b, the mRNA expression levels of the seven electron transport chain genes increased after the SP600125 treatment, which include the mitochondrial NADH dehydrogenase (ND) complex I subunit genes (ND1, ND2 and ND5), Fe-S protein 8 (NDUFS8), Cytochrome C-related genes (NDUFA4 and UQCR) and Succinate dehydrogenase (SDHD). It is noted that except for ND1, the expression levels of the other six electron transport chain-related genes were prominently downregulated at 48 h after SP600125 treatment. Nevertheless, the mRNA expression levels of the seven electron transport chain-related genes were upregulated at 24 h (Fig. 5C).

4. Discussion

The SP600125-treated cells have displayed significantly cell number decreased and some structural changes, for example, the endoplasmic reticulum spread (Fig. 1b, f). These results indicate that the SP600125 has toxic effect on fish cells. However, there are still a number of cells that survive. And in addition to ploidy changes, there are some positive changes in the SP600125-treated cells, such as the increase of cell secretory hyperactivity (Fig. 1d, f). Acute immune stress can affect cell's capacity of resistance to possible damages through generating an inflammatory cell microenvironment, with which a variety of cytokines are involved, such as the inflammatory factors, the adhesion molecules,

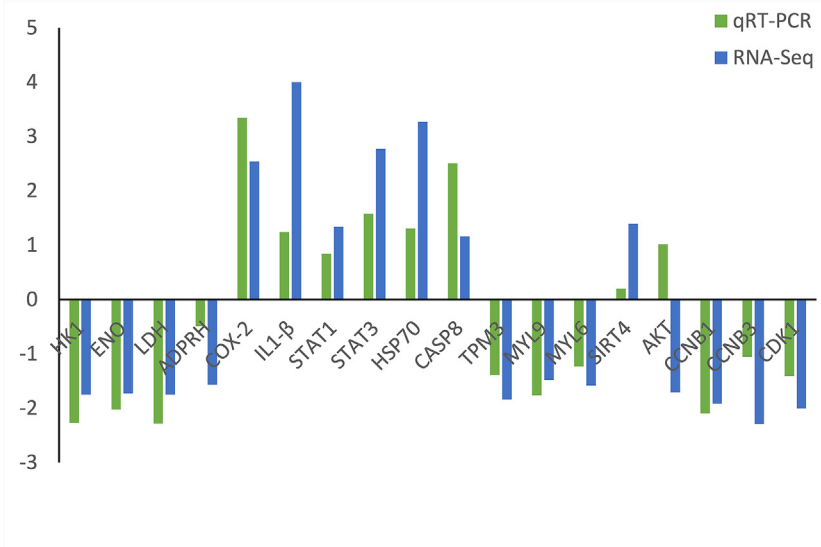

Fig. 3. Comparison between the selected differently expressed genes by qRT-PCR and those from the RNA-seq data in the SP600125-treated cells and in the control cells of crucial carp. The changes of the log-fold represent the ratio of gene expression after normalization to β-actin. For each sample, the qRT-PCR analysis was performed on three biological replicates.

Table 1
The immune-related differently expressed genes in the SP600125-treated cells.

Accession number	Gene ID	Gene name	SP600125-treated VS control		note
			log2 fold Change	adjusted P-value	
cytokines and cytokine receptors	c109435_g1	IL-1β	4	1.33E-11	interleukin-1β
	c20090_g1	TNF-α	3.51	3.40E-13	tumor necrosis factor-α
	c114328_g1	IL-8	2.75	1.36E-04	interleukin- 8
	c89247_g2	IL-11	1.46	2.74E-06	interleukin −11
	c74954_g1	CD40	3.39	1.77E-16	cluster of differentiation 40
	c85599_g1	INF-α	1.3	3.39E-02	interferon–α
	c95368_g1	IL-6R	2.42	1.80E-05	interleukin 6 recepor
	c31247_g1	DOCK2	2.51	9.23E-05	dedicator of cytokinesis-2
	c61123_g1	IL-2R	2.52	8.26E-06	interleukin-2 receptor
adaptor and signal transducers	c91741_g1	STAT1	7.03	1.56E-41	significance of transcriptional activator −1
	c87497_g1	IκBα	1.54	3.53E-06	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
	c89776_g1	STAT3	2.77	6.04E-20	significance of transcriptional activator −3
	c92422_g2	A20	1.99	2.98E-06	zinc finger proteinA20
	c79917_g1	HSP70	3.27	2.84E-08	heat shock protein −70
	c33003_g1	MHC- II	3.59	2.03E-12	major histocompatibility complex class- II
cell adhesion molecules and Integrins	c125550_g1	α2β1	2.26	5.52E-05	integrin α2β1
	c11653_g1	αMβ2	2.35	1.82E-04	integrinαMβ3 receptor
	c88070_g1	VCAM-1	2.11	5.81E-07	vascular cell adhesion molecule-1
	c68540_g1	TSP	4.57	1.84E-24	thrombospondin
	c11653_g1	ITGB2	2.35	1.82E-04	integrin beta −2
	c181667_g1	αVβ3	2.71	7.47E-07	integrin αVβ3
chemokines and chemokine receptors	c80826_g1	CXCR3	1.29	1.00E-03	CXC chemokine receptors-3
	c72678_g1	CXCR4	3.97	5.28E-06	CXC chemokine receptor-4
	c173654_g1	CXCL11	4.16	1.51E-04	C-X-C motif chemokine −11
TCR signaling pathway	c32302_g1	CD8	2.92	1.05E-05	cluster of differentiation −8
	c79421_g2	CD9	5.71	3.24E-42	cluster of differentiation −9
inflammatory inducible enzyme	c83676_g2	COX-2	4.58	6.12E-28	cyclooxygenase-2
apoptosis-related genes	c85181_g2	CASP8	1.16	8.84E-05	caspases-8

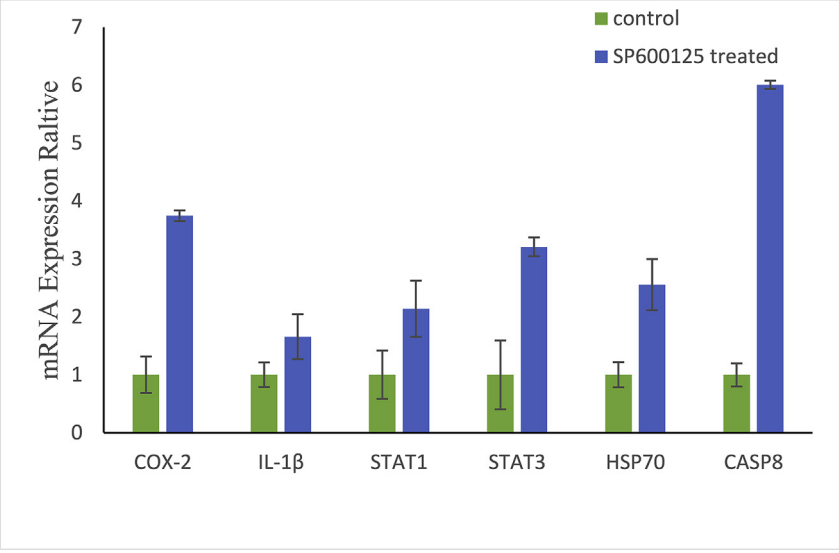

Fig. 4. Comparison between the SP600125-treated cells and those in the control group for the six immune-related differently expressed genes by qRT-PCR, where β-actin was used as an internal control (mean ± SD of relative expression). For each sample, the qRT-PCR analysis was performed on three biological replicates.

and the chemokines [26–28]. Therefore, in the SP600125-induced polyploidy of fish cells *in vitro* obtained from our previous research, it is an interesting issue to address what are the specific changes of the above mentioned cytokines as the fish cells are treated by SP600125.

Our findings in this study have demonstrated that the SP600125-stress can affect the intracellular pathways and regulate the cellular homeostasis feedback. Actually, a number of differentially expressed genes were found to be involved in innate defense, inflammatory pathways and cell adhesion molecules-related pathways. In addition, SP600125-stress can also enhance the cell-mediated immunity, which has been characterized by the significantly increased expression levels of multiple immune genes.

Specifically, it was suggested that COX-2 plays a critical role in the pathogenesis of inflammation as it is rapidly expressed in response to a diverse range of proinflammatory mediators and cytokines [29,30]. Like NF-KB and AP-1, COX-2 exerts anti-inflammatory and anti-oxidative effects [31]. Another inflammatory cytokines, IL-β, was regarded to be a potential inflammatory mediator in response to infection and injury, and was only synthesized under the conditions of cell stress or pathogenic invasion [32]. In addition, JAK/STATs have been verified

Table 2
The mitochondria-related differently expressed genes of SP600125-treated cells.

Gene ID	Gene name	SP600125 treated VS control		note
		log 2 fold Change	adjusted P-value	
c72228_g2	TOM6	1.05	2.14E-02	mitochondrial import receptor subunit TOM6 homolog
c83381_g1	SLC2A3	−3.75	2.76E-05	solute carrier family 2, facilitated glucose transporter member 3
c71899_g1	GLUT1	−2.01	2.85E-02	glucose transporter 1
c92886_g1	HK1	−1.75	3.88E-02	hexokinase 1
c79994_g1	PFKFB	−1.52	1.38E-03	6-phosphofructo-2-kinase/fructose-2
c87360_g1	ENO	−1.73	4.09E-02	enolase
c83242_g3	LDH	−1.75	4.04E-02	ldha protein
c81649_g1	GLUD1_2	−2.13	1.38E-04	glutamate dehydrogenase 1b
c61198_g1	COX8	−3	6.98E-03	cytochrome c oxidase subunit 8B
c71395_g1	COX5B	−2.39	2.21E-03	lytochrome c oxidase subunit 5B
c68072_g1	COQ5	1.02	2.71E-02	Ubiquinone biosynthesis methyltransferase COQ5
c88578_g1	CPOX	−2.28	8.99E-03	coproporphyrinogen oxidase
c87958_g5	GOT2	−1.71	2.35E-06	glutamic-oxaloacetic transaminase 2
c50795_g1	CLU1	3.65	2.78E-05	clustered mitochondria protein homolog isoform X1
c84681_g2	ADHFE1	−1.01	2.15E-02	alcohol dehydrogenase, iron containing, 1
c89985_g1	SARDH	−1.61	7.71E-05	sarcosine dehydrogenase
c86324_g1	CMPK2	4.29	4.29E-22	UMP-CMP kinase 2
c85600_g2	ADK	−1.47	1.58E-04	adenylate kinase isoenzyme 4
c70046_g1	UCP4	1.36	4.09E-02	mitochondrial uncoupling protein 4
c54596_g1	SLC25A2_15	1.86	2.22E-02	solute carrier family 25
c89517_g2	CTP	−2.07	2.87E-05	tricarboxylate transport protein
c91100_g1	GPAT1_2	−1.43	3.00E-02	glycerol-3-phosphate acyltransferase 1
c80940_g1	GATM	2.89	5.66E-11	glycine amidinotransferase
c86334_g1	OXCT	−2	1.42E-03	3-oxoacid CoA transferase 1a
c92589_g1	NNT	−1.25	4.63E-03	NAD(P) transhydrogenase
c5208_g1	TDH	−1.84	1.25E-02	L-threonine 3-dehydrogenase
c89523_g1	SDHA	1.09	5.83E-03	succinate dehydrogenase
c93239_g1	Pyc	−1.52	5.65E-05	pyruvate carboxylase
c83446_g2	MSRa	2.13	2.89E-08	peptide methionine sulfoxide reductase
c172833_g1	GPX4	1.94	7.55E-03	glutathione peroxidase 4 precursor
c60280_g1	ENDOG	−1.4	2.18E-03	endonuclease G

to be involved in the regulation of viral infections and cellular damage [33,34]. HSPs were shown to be a group of conserved proteins induced in prokaryotes and eukaryotes by numerous types of cellular stresses, and were identified that it (HSP70) could help cells resist deadly conditions and adapt to stress [35–37]. Caspases, which are involved in extrinsic apoptotic signaling pathways and stimulate various cell receptors to initiate apoptosis, were regarded to be key regulators of inflammatory and innate immune responses [38–41].

Actually, in the SP600125-treated cells, many of the highly upregulated DEGs were the innate defense molecules, such as interleukins and interleukin receptors, complement components, and chemokines and chemokine receptors, in comparison with the control ones. As shown in Ref. [42], it is further revealed that the above mentioned genes are responsible for promoting cell adaptation downstream to stress response pathways. One of main results in this study lies in that the SP600125-stress can improve cell resistance to chemical poison damage in SP600125-induced polyploidy of fish cells *in vitro* by generating suitable microenvironment balance of cell survival.

As one of the most important organelles in eukaryotic cells, the mitochondria are also contributed to cellular homeostasis regulation upon SP600125 exposure. The effects of SP600125 treatment were accompanied by an ATP content decrease in the cells. The decreasing mRNA expression level of GLUT1, and the key enzymes, HK1, ENO and LDH, may directly generate an effect on cell metabolism, which leads to the change of ATP content. The glycolytic pathway begins at the cell membrane as glucose enters the cell through GLUTs such that pyruvic acid was generated. Hexokinases phosphorylate glucose to produce glucose-6-phosphate (G6P), which is the first step in most glucose metabolism pathways. LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back. The lactate-forming reaction generates cytosolic NAD^+, which feeds into the glyceraldehyde 3-phosphate dehydrogenase reaction to help maintain the cytosolic redox potential and promote substrate flux through the second phase of glycolysis, to promote ATP generation [43–46]. Our data furthermore confirmed the change in the mitochondrial electron transport respiratory chain complex-related genes, such as ND1, ND2, ND5, NDUFS8, NDUFA4, SDHD and UQCR, which increased after SP600125 treatment. The gene expression profile reflects the level of cell energy metabolism under the SP600125-stress conditions. These results have demonstrated that during SP600125 exposure, cells may still adjust and enhance the mitochondria function by upregulating expression of the electron transfer chain-related genes, as well as increasing the number of mitochondria.

5. Conclusions

On basis of the RNA-seq transcriptome profiling data of fish cells for identifying host determinants of the response to SP600125 treatment *in vitro*, we have found that the abundant DEGs are involved in the activation of molecular stress response to the SP600125-stress. Such stress has enhanced the expression and secretion of pro-inflammatory cytokines. The changes of gene expression levels can maintain dynamic homeostasis in SP600125-treated cells. In addition, it has also been revealed that the increasing acute mitochondrial stress is contributed to the cellular homeostasis regulation upon the SP600125-stress. These results have shown that acute inflammation is an adaptive and controlled response to the SP600125-stress, which is beneficial for alleviating toxicity by SP600125.

The results in this study can provide a potential way of breeding fish polyploidy induced by SP600125 in the future research.

Competing financial interests

The authors declare no competing financial interests.

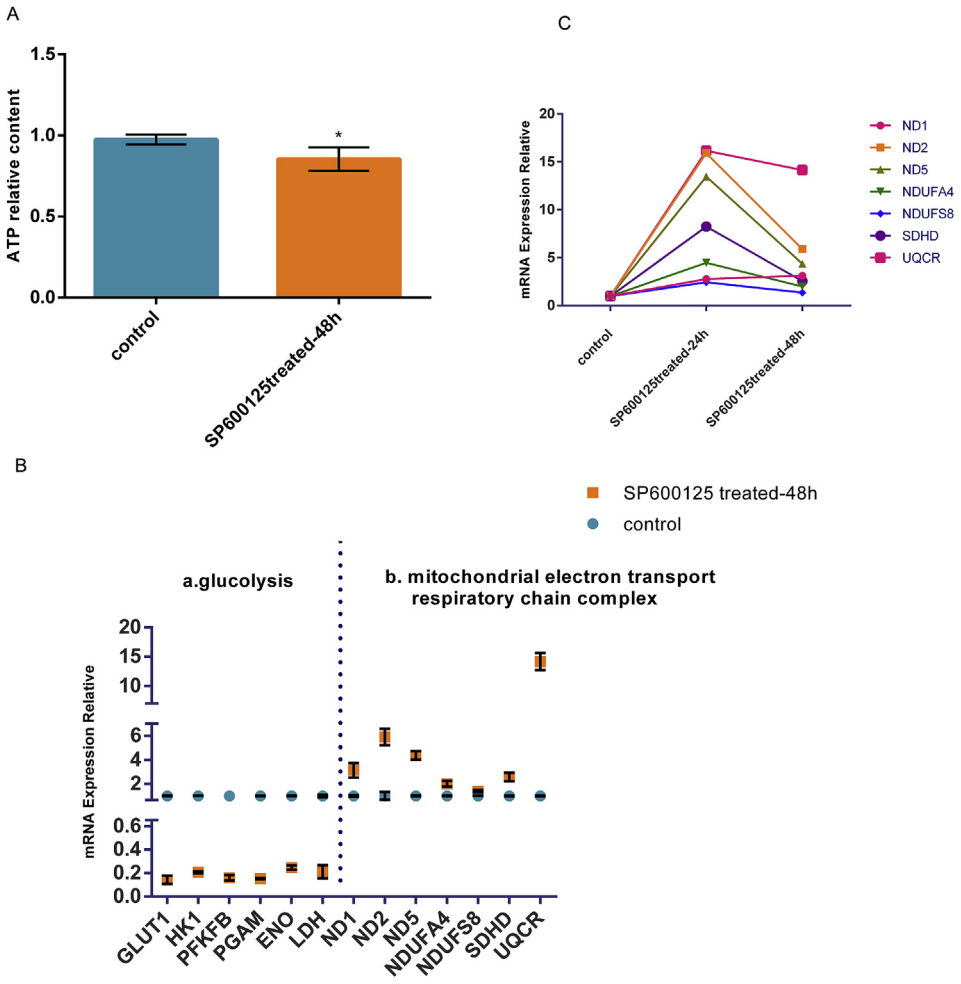

Fig. 5. Analysis of mitochondria in the SP600125-treated cells. (A) ATP content analysis of the SP600125-treated cells and those in the control group (mean ± SD of relative expression). For each sample, this analysis was performed on three biological replicates. * stands for the test level: $P < 0.05$. (B) The expression levels of the six glycolysis genes (a) and those of the seven mitochondrial electron transport respiratory chain complex genes (b) detected by qRT-PCR in the control cells (blue) and in the SP600125-treated ones in 48 h (orange). (C) The mRNA expression levels of the seven electron transport chain genes in the crucial carp cells exposed to 100 μM SP600125 in 0 h, 24 h and 48 h, respectively. For each sample, the qRT-PCR analysis was performed on three biological replicates, where β-actin was used as an internal control (mean ± SD of relative expression). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2018.10.063.

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