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Bioinformatics Analysis Identified the Key Genes of Aspirin and Redox Damaged Yeast Cells

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HYC and PCY conceived and designed the experiments. PCY analyzed the data. HYC contributed materials/analysis tools. PCY wrote the manuscript. YNL, HYL and YS revised the manuscript.

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Aspirin, *Saccharomyces cerevisiae*, Bioinformatics analysis, Functional enrichment analysis, Protein-protein interaction (PPI) network

ABSTRACT

Aspirin is a widely used anti-inflammatory and antithrombotic drug that exhibits chemopreventive anti-tumor properties. Aspirin is considered to be partially mediated by the induction of apoptosis in cells. However, the underlying molecular mechanisms of aspirin in the prevention of cancer are yet to be fully elucidated. In the current study, the GSE115660 microarray dataset was downloaded from the Gene Expression Omnibus database to identify key genes in aspirin-damaged yeast cells following redox injury. Differentially expressed genes (DEGs) were subsequently identified and functionally enriched for analysis. Additionally, a protein-protein interaction network (PPI) was constructed and block analysis was performed using STRING and Cytoscape databases. A total of 248 genes were identified, of which 84 were downregulated and 164 were upregulated. Functional and pathway enrichment analyses indicated that upregulated genes were significantly involved in pyrimidine metabolism, glyoxylic acid metabolism and dicarboxylic acid metabolism. Downregulated genes were primarily implicated in secondary metabolite biosynthesis, carbon metabolism and antibiotic effects. The results revealed that the substance exhibited abundant biosynthesis and glycolysis/gluconeogenesis. Subsequently, the following top 10 hub genes were identified to the PPI network: Guanine nucleotide-binding protein subunit β, ribosomal 60S subunit protein (RPL) 8A, RPL9A, RPL6B, ribosomal 40S subunit protein (RPS) 9B, RPL31B, RPL27B, RPS14B, RPL22B and RPL22A. In conclusion, the DEGs and Hub genes identified in the present study may further elucidate the molecular mechanisms of aspirin applied to redox-damaged yeast cells and may identify potential future biomarkers.

INTRODUCTION

Acetylsalicylic acid (aspirin) is an anti-inflammatory, cardio protective and antithrombotic drug, which has been recently identified to exhibit chemo preventative and anti-tumor properties. These are not only due to the anti-inflammatory and anti-thrombocytic effects of aspirin, but also due to their tendency to cause programmed cell death (Chan *et al*., 2012; Janke *et al*., 2004) in cancer cells. Aspirin induces esophageal cancer cell apoptosis by inhibiting the downstream regulation of cyclooxygenase-2 via nuclear factor-κB (Liu *et al*., 2005). Additionally, TGF-β1 mediates the effect of aspirin on colon cancer cell proliferation and apoptosis (Wang *et al*., 2018b). Aspirin also induces apoptosis and inhibits the proliferation of breast cancer cells (Wu *et al*., 2017). The overall mechanism of aspirin-induced malignant cell death is not fully understood and numerous studies have constructed several experimental models using eukaryotic cells for further elucidation. One of these models utilizes *Saccharomyces cerevisiae*, which has been previously used to study apoptosis in living organisms as cells exhibit various essential eukaryotic processes, including apoptosis brand characteristics (Eisenberg *et al*., 2007). Yeast is also a particularly important tool for the assessment of cell death associated with mitochondria.

Over the past few decades, microarray and bioinformatics analyses have been widely used to detect genetic changes on a genome-wide scale, facilitating the identification of differentially expressed genes (DEGs) in redox-affected yeast cells. In the present study, correlation analysis was performed between the control and experimental groups. Furthermore, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses, along with PPI network analysis were utilized to identify the key genes of

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aspirin-damaged yeast cells to determine the mechanisms underlying cell death.

MATERIALS AND METHODS

Gene expression profile data

The Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) (Edgar *et al*., 2002) database from the National Center for Biotechnology Information stores curated gene expression datasets, original series and platform records. The current study downloaded the GSE115660 gene expression dataset (Farrugia *et al*., 2019) from GEO (Affymetrix GPL2529 platform; Affymetrix Yeast Genome 2.0 Array). Probes were converted to the corresponding gene symbol based on the annotation information provided by the platform. The GSE115660 dataset contained 6 processed samples and 6 normal samples. Subsequently, the differentially expressed genes (DEGs) of the overlap region in the sample were determined using the online tool Venny 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). A heat map of DEGs was constructed using TBtools 0.665 (https://github.com/CJ-Chen/TBtools).

Screening of DEGs

DEGs were screened using the dialog network tool, GEO2R (www.ncbi.nlm.nih.gov/geo/geo2r). This allowed for the identification of DGEs in experimental conditions by comparing >2 datasets of the GEO series. Within the GEO2R platform, samples were grouped and differentially analyzed for grouped data. The genes with significant differences were selected according to an automated design. The P-value and Benjamini and Hochberg false discovery rate was used for the identification of statistically significant genes and false positives, respectively. Genes with one or more probe sets and probe sets without gene symbols were excluded. The screening conditions were as follows: Gene differences of 2 times or more were considered statistically significant (Log_2 Fold Change>1 and P-<0.01).

DEG GO and KEGG pathway enrichment analyses

DAVID (http://david.ncifcrf.gov, version6.8) (Huang *et al*., 2007; Jiao *et al*., 2012) is a bioinformatics database that integrates biological data with analytical tools to provide comprehensive bio-functional annotations for large-scale gene or protein lists to extract biological information. GO (Ashburner *et al*., 2000) is an important bioinformatics tool used for parsing genes and determining the biological processes they are involved in. KEGG (Kanehisa, 2002) is a database that is used to understand advanced functions and biological systems from large-scale molecular data generated by high performance experimental techniques. The up and downregulated genes identified in the current study were uploaded to the online bioinformatics analysis tool, DAVID, to identify the most significantly enriched biological classifications and annotations. P<0.05 was considered to indicate a statistically significant difference.

Protein-protein interaction (PPI) network analysis

STRING (http://string-db.org, version 11.0) (Szklarczyk *et al*., 2019) is a commonly used PPI database, which includes information regarding direct interactions between proteins, as well as correlations of indirect protein functions. Each point in the interaction network represents a protein and each edge represents an interaction between two linked proteins. The DEGs obtained during the present study were mapped on the STRING database to determine the interactions between proteins encoded by the aforementioned genes and to further understand the specific functions of each DEG. For greater biological significance, only the nodes with an interaction score >0.4 were reserved. Due to the complexity of the PPI network, Cytoscape was used to analyze interactions. Cytoscape (Version 3.7.1) (Politano *et al*., 2016) is a visual bioinformatics software that integrates biological networks with various molecular states, including gene expression and genotype. The MCODE (version 1.5.1) (Bandettini *et al*., 2012) plugin was used to cluster functional blocks in a large gene (protein) network. The current study therefore used Cytoscape to draw a PPI network and MCODE to identify the most significant modules of the PPI network. Am MCODE score >10 was used as the screening criterion. Other parameters were selected as the default values. Subsequently, functional enrichment analysis of the genes in the module was performed using DAVID. The key targets and subnets of complex networks were explored using the CytoHubba (Chin *et al*., 2014) (version 0.1) plugin. Among them, MCC is a newly proposed method. Additionally, the prediction of key proteins is more effective in yeast PPI networks compared with other methods. Thus, the current study utilized the MCC method to select the top 10 nodes as hub genes and the remaining parameters were selected as default values.

RESULTS

Identification of DEGs

Following the standardization of GSE115660, DEG analysis was performed. The results revealed a total of 248 DEGs, of which 164 were upregulated and 84 were downregulated, as presented in the Venn diagram (Santos *et al*., 2018) (Fig. 1). The DEG expression heat map of the GSE115660 dataset constructed using TBtools (Chen *et al*., 2018a) is presented in Figure 2. The 248 DEGs were used

for further analysis.

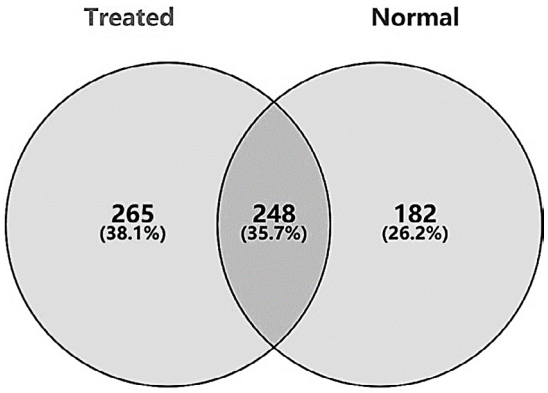

Fig. 1. Venn diagram of 248 DEGs from treated and normal.

DEG functional and pathway enrichment analyses

DEGs were classified via GO analysis and the results elucidated gene function. DEG KEGG analysis determined the differential pathways of samples and identified the changes in certain cellular pathways. The DEGs of the current study were uploaded to DAVID for pathway enrichment analysis and functional annotation. The results revealed that the upregulated DEGs were significantly enriched in the propionate catabolism process, the 2-methyl citrate cycle, the UMP biosynthesis process, the positive regulation of spindle pole separation, the G2/M conversion of the mitotic cell cycle and the pyrimidine nucleotide biosynthesis process. In addition, downregulated DEGs were significantly enriched in the mitochondrial redox process, cytoplasmic translation and pentose phosphate shunt. In terms of cell components, upregulated DEGs were significantly enriched in the plasma membrane and actin. However, downregulated DEGs were enriched in cell walls, ribosomes, fungal vacuoles and within the cytoplasm. For molecular function, upregulated DEGs enhanced transferase activity and the transfer of an acyl group to an alkyl acyl group, releasing two ions during the transfer process and binding two sulfur clusters. Downregulated DEGs significantly enhanced oxidoreductase activity and transporter activity. KEGG pathway analysis revealed that upregulated DEGs were mainly enriched in pyrimidine metabolism, glyoxylic acid metabolism and dicarboxylic acid metabolism. The downregulated DEGs were primarily enriched in the biosynthesis of secondary metabolites, carbon metabolism, the biosynthesis of antibiotics and glycolysis/gluconeogenesis. The enrichment analyses (Tables I and II) of gene functions performed in the present determined the biological pathways that serve a key role in various biological processes, providing further understanding of the basic molecular mechanisms involved.

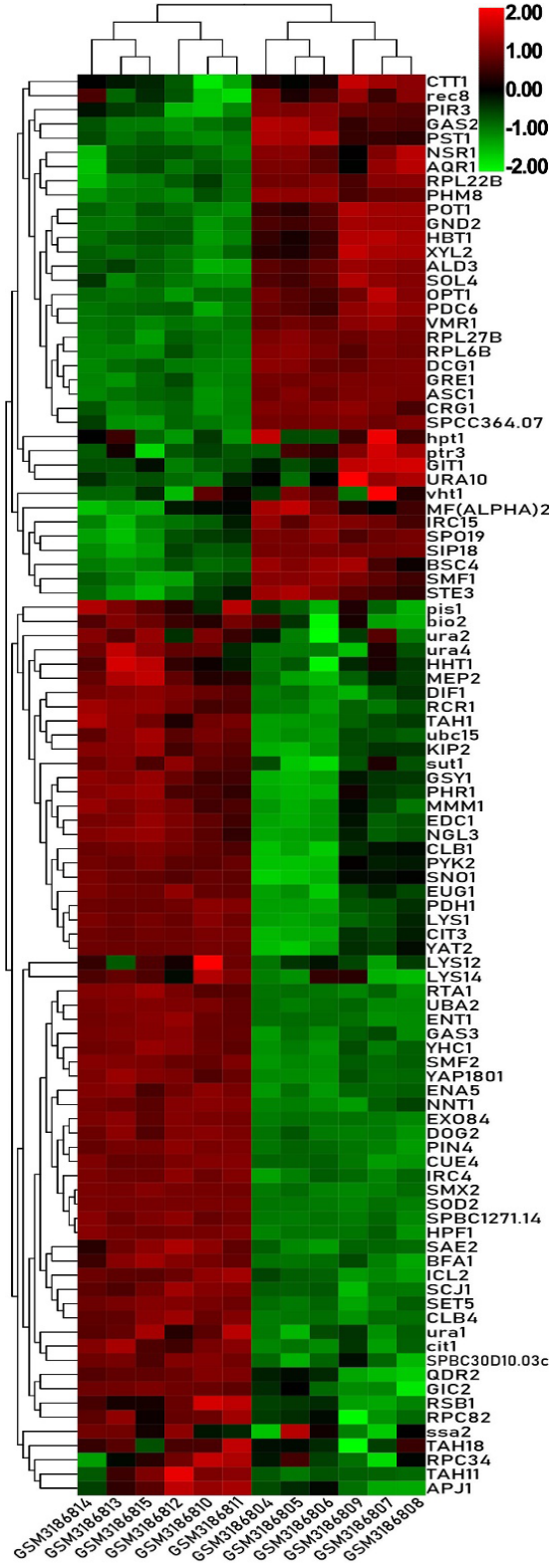

Fig. 2. Heat map of the top 100 differentially expressed genes of GSE115660.

Table I. Result of GO and KEGG enrichment analysis of up-regulated DEGs.

Term	Description	Count	%	P-Value
BP				
GO:0019629	Propionate catabolic process, 2-methylcitrate cycle	3	2.0	1.37E-03
GO:0044205	'De novo' UMP biosynthetic process	3	2.0	6.55E-03
GO:0010696	Positive regulation of spindle pole body separation	3	2.0	9.05E-03
GO:0000086	G2/M transition of mitotic cell cycle	4	2.6	9.83E-03
GO:0006207	'De novo' pyrimidine nucleobase biosynthetic process	3	2.0	1.51E-02
GO:0006221	Pyrimidine nucleotide biosynthetic process	3	2.0	1.86E-02
CC				
GO:0005887	Integral component of plasma membrane	8	5.3	2.13E-02
GO:0005886	Plasma membrane	19	12.5	2.36E-02
GO:0030479	Actin cortical patch	5	3.3	4.66E-02
MF				
GO:0046912	Transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer	3	2.0	1.39E-02
GO:0051537	2 ion, 2 sulfur cluster binding	3	2.0	4.21E-02
KEGG				
sce00630	Glyoxylate and dicarboxylate metabolism	4	2.6	1.03E-02
sce00240	Pyrimidine metabolism	5	3.3	3.42E-02
sce01200	Carbon metabolism	6	3.9	4.94E-02

Construction of PPI network and module analysis

Protein interaction networks are crucial for understanding the reaction mechanisms of biological signals and energy metabolism under specific physiological conditions, including diseases, and for understanding the functional links between proteins. STRING was utilized to build the DEG PPI network. Cytoscape was then utilized to detect the most significant modules. Functional enrichment analysis of the genes involved in these modules was performed using DAVID. The DEG PPI network is presented in Figure 3. The network contained 374 nodes and 1,218 edges. The most important modules obtained from the PPI are presented in Figure 4. Table III summarizes the functional enrichment analysis of the most important modules obtained using MCODE. The top 10 central nodes with higher degrees selected from the PPI network are presented in Figure 5. The results revealed that the 10 hub genes obtained using CytoHubba were identical to module 2 of MCODE, confirming the credibility of identified hub genes. The following hub genes were identified: Guanine nucleotide-binding protein subunit β (ASC1), ribosomal 60S subunit protein (RPL) 8A, RPL9A, RPL6B, ribosomal 40S subunit protein (RPS) S9B, RPL31B, RPL27B, RPS14B, RPL22B and RPL22A.

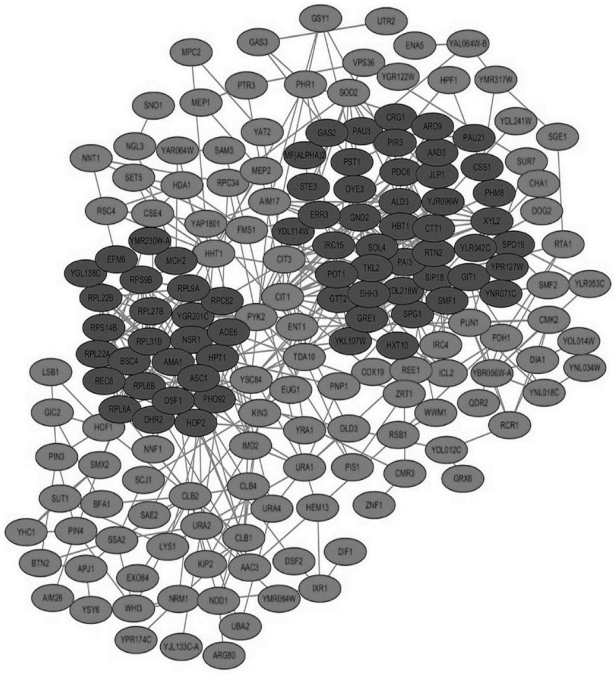

Fig. 3. Protein-protein interaction network diagram. Red indicates the up-regulated gene and green indicates the down-regulated gene.

Table II. Result of GO and KEGG enrichment analysis of down-regulated DEGs.

Term	Description	Count	%	P-Value
BP				
GO:0055114	Oxidation-reduction process	13	15.7	4.97E-04
GO:0002181	Ccytoplasmic translation	8	9.6	3.27E-03
GO:0006098	Pentose-phosphate shunt	3	3.6	1.24E-02
GO:0006412	Translation	9	10.8	2.84E-02
CC				
GO:0022625	Cytosolic large ribosomal subunit	7	8.4	1.98E-03
GO:0000324	Fungal-type vacuole	11	13.3	2.26E-03
GO:0009277	Fungal-type cell wall	7	8.4	2.61E-03
GO:0031225	Anchored component of membrane	5	6.0	8.06E-03
GO:0005618	Cell wall	5	6.0	9.44E-03
GO:0005840	Ribosome	10	12.0	1.08E-02
GO:0005622	Intracellular	8	9.6	1.18E-02
GO:0005737	Cytoplasm	39	47.0	1.84E-02
GO:0030529	Intracellular ribonucleoprotein complex	9	10.8	3.46E-02
GO:0005576	Extracellular region	5	6.0	4.56E-02
MF				
GO:0016491	Oxidoreductase activity	13	15.7	1.47E-04
GO:0003735	Structural constituent of ribosome	9	10.8	1.52E-02
GO:0005199	Structural constituent of cell wall	4	4.8	1.66E-02
GO:0005215	Transporter activity	5	6.0	1.81E-02
GO:0008757	S-adenosylmethionine-dependent methyltransferase activity	3	3.6	2.84E-02
KEGG				
sce03010	Ribosome	9	10.8	1.79E-03
sce01110	Biosynthesis of secondary metabolites	11	13.3	3.24E-03
sce01130	Biosynthesis of antibiotics	9	10.8	5.35E-03
sce00010	Glycolysis / Gluconeogenesis	4	4.8	3.98E-02

DISCUSSION

By analyzing microarray data, the present study identified the DEGs of aspirin-injured yeast cells, of which 164 were upregulated and 84 were downregulated. A series of bioinformatics analyses, consisting of GO terminology analysis, KEGG pathway analysis and PPI network analysis, were performed to screen hub genes and the pathways closely associated with aspirin-infected yeast cells.

Table III. Functional and pathway enrichment analysis of the genes in the most significant modules.

Term	Description	Count	FDR
Module 1			
GO:0006098	Pentose-phosphate shunt	3	2.42E-04
GO:0009051	Pentose-phosphate shunt, oxidative branch	2	8.07E-03
GO:0055114	Oxidation-reduction process	4	1.53E-02
GO:0005737	Cytoplasm	10	1.66E-03
GO:0016491	Oxidoreductase activity	4	6.18E-03
sce01110	Biosynthesis of secondary metabolites	5	3.27E-04
sce01200	Carbon metabolism	4	5.34E-04
sce00030	Pentose phosphate pathway	3	9.36E-04
sce01130	Biosynthesis of antibiotics	4	3.49E-03
Module 2			
GO:0002181	Cytoplasmic translation	8	7.91E-10
GO:0006412	Translation	9	9.05E-10
GO:0042254	Ribosome biogenesis	3	0.038916
GO:0005840	Ribosome	9	3.81E-10
GO:0030529	Intracellular ribonucleoprotein complex	9	4.70E-10
GO:0022625	Cytosolic large ribosomal subunit	7	2.74E-09
GO:0005622	Intracellular	7	1.24E-07
GO:0005737	Cytoplasm	10	2.59E-04
GO:0022627	Cytosolic small ribosomal subunit	3	0.005067
GO:0003735	Structural constituent of ribosome	9	6.97E-10
GO:0003723	RNA binding	5	0.007587
sce03010	Ribosome	9	1.93E-09

The aim of the present study was to determine the mechanism of aspirin-induced apoptosis at the molecular level in redox-damaged *Saccharomyces cerevisiae* cells. The current study identified the DEGs of MnSOD-deficient *Saccharomyces cerevisiae* cells exposed to aspirin-induced redox damage and wild-type yeast cells. Aspirin alters the gene expression profile of redox-damaged MnSOD deficiency, resulting in a larger and more significant gene

expression profile, which is significantly different from wild-type yeast cells (Holley *et al*., 2013; Oberley and Buettner, 1979).

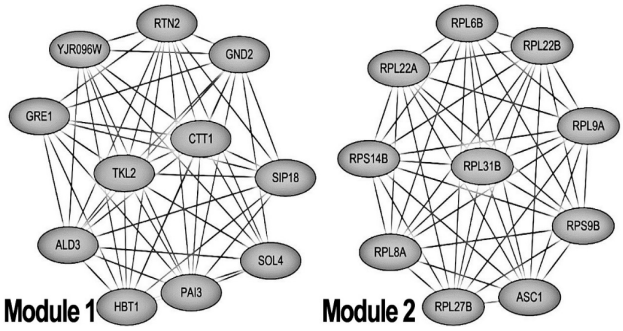

Fig. 4. The most significant modules obtained from the PPI network. Module 1 and module 2 are the most important modules in the PPI network identified by the MCODE score > 10.

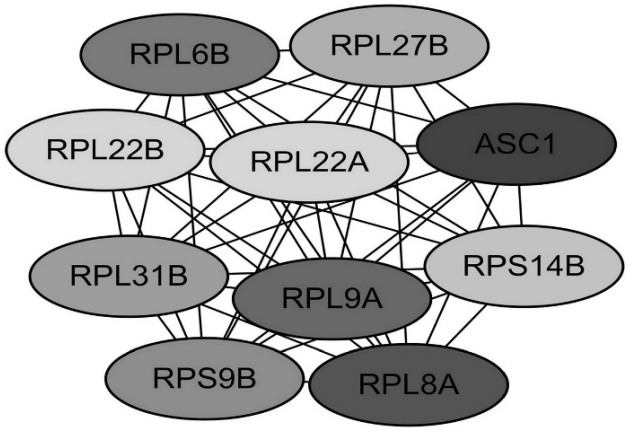

Fig. 5. Functional roles of 10 hub genes with MCC ≥10. The darker the color, the higher the score.

The extent to which aspirin affects mutant and wild-type gene expression profiles can be explained by the lack of the mitochondrial antioxidant enzyme, MnSOD, in mutant yeast cells, resulting in different gene expression profiles (Qin *et al*., 2013). Since genotypes have been reduced, certain adaptations in the mutated transcripts of these genotypes involve the development of sensitivity to aspirin in defective mutant yeast cells. Additionally, aspirin further exacerbates their downregulation.

The functional annotation (GO) and pathway enrichment (KEGG) analyses of the present study indicated that downregulated DEGs were primarily enriched in various ribosome-associated components, including "cell ribonucleoprotein complex", "cytoplasmic ribosomal subunit" and "cellular ribonucleoprotein complex". KEGG pathway analysis determined that the DEGs were mainly enriched in secondary biosynthesis, biosynthesis of metabolites and antibiotics. The results indicated that aspirin inhibited ribosome synthesis, limiting the ability of cells to produce protein (Chen *et al*., 2018b).

The role of ribosomes in the regulation of gene expression is complex and changes in protein components in response to changes in the growth environment in vivo (Morris, 2008). The top 10 proteins of the PPI network constructed in the present study were involved in ribosome regulation and were all downregulated. ASC1 triggers a series of reactions to stop and prevent ribosome translation (Wang *et al*., 2018a). The downregulation of RPS14B affects ribosome assembly (Fewell and Woolford, 1999) and RPS9B inhibits ribosome translation (Plocik and Guthrie, 2012). RPL22A and RPL22B (Kim and Strich, 2016) affects the fate of yeast cells, and the reduction of RPL22 inhibits pseudohyphal growth, resulting in false defects of meiotic operation. Low expressions of RPL9A and RPL8A inhibit protein expression in *Saccharomyces cerevisiae* (Lee and Stevens, 2016). RPL27B affects the assembly of the ribosomal gene protein regulatory elements (Kasahara *et al*., 2007). In addition, low expressions of RPL31B (Enyenihi and Saunders, 2003; Peisker *et al*., 2008; Winzeler *et al*., 1999) affects ribosome function and may be involved in the normal function of the molecular chaperone complex. The downregulation of RPL6B (Moritz *et al*., 1990) also disrupts the composition of the ribosome and the sequences assembled by the ribosomal protein.

CONCLUSIONS

In summary, 248 DEGs were identified and 10 hub genes were listed as potential biomarkers via bioinformatics analysis. An in-depth understanding of the molecular mechanisms underlying aspirin-induced *Saccharomyces cerevisiae* was gained. Although the present study is preliminary, the results may have potential for future clinical applications and as such require validation by further experimental studies.

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Statement of conflict of interest

The authors have declared no conflicts of interest.

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