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Research Article

Transcriptomic profiling of chemical exposure reveals roles of Yap1 in protecting yeast cells from oxidative and other types of stresses

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Abstract

Transcriptomic profiles are generated by comparing wild-type and the yeast *yap1* mutant to various chemicals in an attempt to establish a correlation between this gene mutation and chemical exposure. Test chemicals include ClonNAT as a non-genotoxic agent, methyl methanesulphonate (MMS) as an alkylating agent, *tert*-butyl hydroperoxide (*t*-BHP) as an oxidative agent and the mixture of *t*-BHP and MMS to reflect complex natural exposure. Differentially expressed genes (DEGs) were identified and specific DEGs were obtained by excluding overlapping DEGs with the control group. In the MMS exposure group, deoxyribonucleotide biosynthetic processes were upregulated, while oxidation–reduction processes were downregulated. In the *t*-BHP exposure group, metabolic processes were upregulated while peroxisome and ion transport pathways were downregulated. In the mixture exposure group, the proteasome pathway was upregulated, while the aerobic respiration was downregulated. Homologue analysis of DEGs related to human diseases showed that many of DEGs were linked to cancer, ageing and neuronal degeneration. These observations confirm that the *yap1* mutant is more sensitive to chemicals than wild-type cells and that the susceptible individuals carrying the *YAP1*-like gene defect may enhance risk to chemical exposure. Hence, this study offers a novel approach to environmental risk assessment, based on the genetic backgrounds of susceptible individuals. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: ***Saccharomyces cerevisiae*; RNA-seq; chemical exposure; risk assessment; YAP1**

Introduction

'Omics' technology has recently become popular in environmental toxicology and human health risk assessment research (Vlaanderen *et al.,* 2010). In the field of toxicology, a new discipline termed 'toxicogenomics', that utilizes high-throughput 'omics' methods and bioinformatics analysis tools to understand the toxicological effects and mechanisms of chemicals, is widely used (Aardema and MacGregor, 2002; Hamadeh *et al.,* 2002a). The main purposes of toxicogenomics are to understand the relationship between toxicological effects and human diseases, obtain sensitive biomarkers of exposure and elucidate mechanisms of toxicity (Waters and Fostel, 2004). Toxicological effects of certain chemicals are associated with changes of gene expression patterns after exposure (Farr and Dunn, 1999). Based on the hypothesis that variations in the gene expression pattern can reflect cellular responses to specific toxic compounds (Dos Santos *et al.,* 2012), numerous toxicogenomics data have been generated in different species (Burczynski *et al.,* 2000; Cui *et al.,* 2007; Yang

et al., 2007; Yasokawa and Iwahashi, 2010). Moreover, it has been reported that compounds with similar toxic mechanism result in similar but distinguishable transcriptional alterations (Hamadeh *et al.,* 2002c; Waring *et al.,* 2001), which makes it possible to utilize chemical-specific gene expression profiles to characterize unknown environmental compounds (Hamadeh *et al.,* 2002b). Thus, by establishing chemical-specific patterns of toxicological model of action, adverse effects could be recognized and the potential hazard of environmental samples could be identified or classified at an early stage (Oberemm *et al.,* 2005). More importantly, with the improved understanding of toxicological effect of exposure chemicals, the specific molecular response may provide valuable information to predict human outcomes (McHale *et al.,* 2014; Singh and Li, 2011; Waters *et al.,* 2003). However, individual susceptibility in genetic background could impact on the evaluation of toxic effects of specific chemicals or environmental samples. Therefore, taking genetic variation into consideration is also an important research strategy for risk assessment of chemical exposure.

The yeast *Saccharomyces cerevisiae* is a preferred model for toxicogenomics study because of its complete genome annotation (Goffeau *et al.,* 1996). Several features have made budding yeast an excellent model system, such as being a unicellular eukaryotic organism, inexpensive, with rapid growth and convenient genetic and high-throughput manipulation (Botstein and Fink, 2011). Furthermore, many pathways and cellular processes involved in toxic responses are highly conserved with human and other higher eukaryotes (Foury, 1997). A variety of genotoxicity test systems have been developed in yeast cells, based on the transcriptional response to DNA damage, such as *RAD54–GFP* and *RNR2–GFP* reporter systems (Afanassiev *et al.,* 2000), the *RNR3–lacZ* reporter system (Jia *et al.,* 2002), the *lexA–GAL4* (Ichikawa and Eki, 2006) and *RNR3–yEGFP* and *HUG1–yEGFP* biosensors (Wei *et al.,* 2013). However, these methods are low throughput and unable to characterize the toxicity mechanism and global cellular effects of chemicals. In order to gain further insights into toxicological response at the genome, transcriptome, proteome and metabolome levels, interdisciplinary toxicogenomics has rapidly been developed. At the transcriptome level, there have been several toxicogenomic reports in yeast using microarrays (Gasch *et al.,* 2001; Jelinsky and Samson, 1999). Recently RNA-seq has became a powerful tool in toxicogenomic research because of its advantages over microarray, including independence of reference genome, lower background noise, broader detection range and higher reproducibility (Wang *et al.,* 2009).

In a previous study (Zhang *et al.,* 2011), we found that deletion of yeast *YAP1* enhances sensitivity of the *RNR3–lacZ* reporter to various DNA-damaging agents. Yap1 belongs to the bZIP family of transcriptional factors that activate most antioxidant genes in response to oxidative stress, including *GSH1* (γ-glutamylcysteine synthetase), *GPX2* (glutathione peroxidase), *TRX2* (thioredoxin) and *TSA1* (thioredoxin peroxidase) (Dumond *et al.,* 2000; Moye-Rowley, 2002). Genes encoding the membrane-associated transporter Ycf1, as well as the multidrug resistance transporter Atr1 and Flr1, are also regulation targets of Yap1 (Alarco *et al.,* 1997; Coleman *et al.,* 1997; Nguyen *et al.,* 2001; Wemmie *et al.,* 1994). Based on *YAP1* functions and our previous observations, we predicted that *YAP1* deletion not only affects *RNR3* expression but also the expression of other genes, many of which are involved in DNA damage and other stress responses. In this study, we used the RNA-seq technology to analyse the transcriptomic response of wild-type and *yap1* mutant strains after exposure to toxic chemicals. The chemicals used here are an alkylating agent, methyl methanesulphonate (MMS), a typical oxidative agent, *tert*-butyl hydroperoxide (*t*-BHP), and a mixture of *t*-BHP and MMS. Genes differentially expressed in wild-type cells and the *yap1* mutant, along with their Gene Ontology (GO) enrichment analysis, KEGG pathway enrichment analysis and the specific expression patterns of each chemical, were analysed. The aims of this study were to generate a specific transcriptome profile after chemical exposure and to establish a correlation between chemical exposure and gene mutation, using yeast as a model.

Materials and methods

Yeast strains, plasmids and transfomation

The haploid *S. cerevisiae* strain BY4741 (*MAT***a** *his3*Δ*0 leu2*Δ*0 met15*Δ*0 ura3*Δ*0*) was created

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by the *Saccharomyces* Gene Deletion Project and was used as a wild-type control; its *yap1* mutant was constructed in a previous study (Zhang *et al.,* 2011). Yeast cells were grown at 30°C in YPD medium. Plasmid DNA was transformed into yeast cells by a modified lithium acetate protocol (Hill *et al.,* 1991) and selected on minimal SD medium. Transformants were streaked on a fresh selective plate before being utilized for further analysis. Plasmid pZZ2 (Zhou and Elledge, 1992) was obtained from Dr S. Elledge (Harvard University, Boston, MA, USA) and utilized for the *RNR3–lacZ* test, as previously described (Jia and Xiao, 2003; Jia *et al.,* 2002). The *RNR3–lacZ* test was used in this study to confirm the DNA damage response induced in yeast cells.

Test chemicals, DNA damage exposure and β-galactosidase (β-gal) assay

MMS and *t*-BHP were purchased from Sigma-Aldrich (St Louis, MO, USA). Non-genotoxic chemical ClonNAT (Nourseothricin) was used as a non-genotoxic control and was purchased from Werner BioAgents (Jena, Germany). All the above chemicals were dissolved in sterile distilled water and stored at 4°C. DNA damage exposure and β-gal assay were performed as described (Jia and Xiao, 2004; Xiao *et al.,* 1993). Briefly, 3 ml overnight yeast culture was used to inoculate fresh SD selective medium until $OD_{600nm} = 0.11$, and incubation was continued for another 2 h. At this point, cell culture always grew up to $OD_{600nm} = 0.14$ and chemicals were added at the concentration indicated, and the cells were incubated for another 4 h. After the incubation, 1 ml of the above unsynchronized log-phase cell suspension was used to determine the cell titre by measuring OD_{600nm}, and 2 ml of the cells were used for the β-gal assay. Yeast cells were precipitated by centrifugation, washed twice with sterile distilled water and resuspended in Z buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgSO_4$ and 40 mM β-mercaptoethanol, pH 7.0) for the β-gal assay, using orthonitrophenyl-β-galactoside as the substrate. The β-gal activity was expressed in Miller units (Guarente, 1983).

Total RNA preparation

After 4 h of exposure, 2 ml cells were used for the β-gal assay, as described above, and 4 ml of the remaining cells/sample were collected for total RNA extraction. Total RNA extraction was performed using an RNA Extraction Kit (Omega Bio-Tek), following the manufacturer's instructions. Total RNA contents were measured using NanoDrop 8000 (Thermo Scientific) and the quality of RNA samples was assessed by agarose gel electrophoresis.

Library construction, clustering and sequencing

cDNA library construction and sequencing were performed by Novogene Co. Ltd, Beijing, China (http://www.novogene.cn/). Before library construction, RNA integrity and concentration were confirmed using a RNA Nano 6000 Assay Kit of the Agilent Bioanalyser 2100 system (Agilent Technologies, CA, USA) and a Qubit RNA Assay Kit in a Qubit 2.0 Fluorometer (Life Technologies, CA, USA). Then a total amount of 3 μg RNA/sample was used as input material for the RNA sample preparations. Sequencing libraries were generated using NEBNext Ultra RNA Library PrepKit for Illumina (NEB, USA), following the manufacturer's recommendations, and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEBNext First Strand Synthesis Reaction Buffer (5×). First-strand cDNA was synthesized using random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-). Second-strand cDNA synthesis was subsequently performed using DNA polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of the 3′ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 150–200 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, CA, USA). Then 3 μl USER Enzyme (NEB, USA) was used with size-selected, adaptor-ligated cDNA at 37°C for 15 min, followed by 5 min at 95°C before PCR. Then PCR was performed, using Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. Finally, the PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyser 2100 system. The clustering

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of the index-coded samples was performed on a cBot Cluster Generation System, using TruSeq SR Cluster Kit v3-cBot-HS (Illumia), according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina Hiseq 2000 platform and 100 bp single-end reads were generated.

Bioinformatic analysis of the RNA-seq data

Raw data of Fastq format were first processed through in-house Perl scripts. In this step, clean data were obtained by removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data. At the same time, the Q20, Q30 and GC contents of the clean data were calculated. All the downstream analyses were based on the clean data with high quality. Reference genome and gene annotation were downloaded directly from the database (http://www.yeastgenome.org/). Single-end clean reads were aligned to the reference genome using TopHat v. 2.0.9 (Trapnell *et al.,* 2009). HTSeq v. 0.5.4p3 was used to count the reads numbers mapped to each gene (Anders, 2010). Then reads per kilobase (RPKM) of each gene was calculated, based on the length of the gene and reads count mapped to this gene. RPKM of exon model per million mapped reads considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels (Mortazavi *et al.,* 2008). Prior to differential gene expression analysis, for each sequenced library the read counts were adjusted by the edgeR program package through one scaling normalized factor (Robinson and Oshlack, 2010). Differential expression analyses of comparison groups were performed using the DEGSeq (Wang *et al.,* 2010) R package v. 1.12.0. The p values were adjusted using the Storey method (Storey and Tibshirani, 2003). Corrected p value of 0.005 and $\log_2$ (fold change) of 1 were set as the threshold for significantly differential expression. Those sequencing data have been deposited in the NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/Traces/sra) and the Accession No. is SRP053279.

Quantitative real-time PCR (qPCR) analysis

qPCR analysis were performed to validate the results from RNA-seq. Total RNA from each sample that shared the same treatment with RNA-seq experiment were reverse transcribed into first-strand cDNA, using oligo (dT) primer with the RevertAid™ First Strand cDNA Synthesis Kit from Thermo. Before qPCR analysis, a standard curve of each primer pair was generated by the regression of Cq values and two-fold cDNA series dilutions from the mixture of all samples. The amplification efficiency of each primer pair was calculated based on the slope of the standard curve. The expression level of *ACT1* and *PDA1* were stable among samples of RNA-seq and were therefore chosen as the internal reference for the normalization of gene expression (Vandesompele *et al.,* 2002).

qPCR analysis was performed in a CFX Connect™ Real-Time PCR Detection System (Bio-Rad). A 2× SYBR Green Real Time PCR Master mix (Toyobo) was used as the reagent mix for qPCR. The qPCR amplification programme was set as follows: 3 min of denaturation at 95°C, followed by 40 cycles of 15 s at 95°C, 15 s at 60°C and 30 s at 72°C. After this procedure, the melt curve of the qPCR product was generated by heating the samples from 65C to 95°C with 0.5°C increments and 5 s plate read time. A single melt peak was considered as a sign of specific amplification. All of these amplification reactions were conducted in triplicate for each gene. The average normalized expression of target genes was calculated using the method of Livak and Schmittgen (Livak and Schmittgen, 2001).

GO and KEGG enrichment analysis of differentially expressed genes

Gene Ontology (GO) and KEGG enrichment analysis of differentially expressed genes were implemented by Bingo v. 2.44 (Maere *et al.,* 2005) and ClueGO v. 1.7.1 (Bindea *et al.,* 2009), plug-ins of Cytoscape v. 2.8.2 (Shannon *et al.,* 2003), in which a hypergeometric test was used to identify enriched GO terms and KEGG pathway terms and the Benjamini–Hochberg method was used for multiple testing correction of the p values (Benjamini and Hochberg, 1995). GO terms and KEGG pathway terms with corrected $p < 0.05$ were considered significantly enriched by differentially expressed genes.

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Results

Validation of DNA damage response induced by chemicals

To confirm whether the yeast samples for transcriptomics analysis had been induced by MMS or *t*-BHP similar to that previously reported, a *RNR3–lacZ* reporter system was introduced to measure the DNA damage response. ClonNAT was used as a non-genotoxic control that may cause a cellular stress response of yeast cells but not a DNA damage response. Test chemical concentrations were chosen based on our previous report (Zhang *et al.,* 2011). Therefore, exposure groups for both wild-type cells and the *yap1* mutant were set as follows: 0.1 mM *t*-BHP, 100 ppm MMS, 20 μg/ml ClonNAT, a mixture of 0.1 mM *t*-BHP and 100 ppm MMS. The control group had the same culture conditions without the above chemical exposure.

After 4 h of exposure, cells of each group were disrupted to measure the *β*-gal activity (Figure 1A). Compared with the wild-type, the *yap1* mutation enhanced 0.1 mM *t*-BHP induced *RNR3–lacZ* expression by nearly six-fold. When exposed to 100 ppm MMS, the *yap1* mutant also displayed an increase in the *RNR3–lacZ* expression by 5.5-fold compared with wild-type cells. As a non-genotoxic agent, 20 μg/ml ClonNAT did not induce *RNR3–lacZ* expression in either the wild-type or the *yap1* mutant. Interestingly, a mixture of 0.1 mM *t*-BHP and 100 ppm MMS dramatically induced *RNR3–lacZ* expression in wild-type cells, while induction in the *yap1* mutant was lower than in wild-type cells. This was probably due to the toxicity of *yap1* mutant by mixed chemical exposure. Indeed, *yap1* mutant cells grew poorly in the presence of both test chemicals, and their toxic effects appeared to be additive (Figure 1B). Overall, the above observations are consistent with our previous studies, and the mixed chemical exposure reflects an unexpected complexity, which allows it to be further explored by global transcriptional response analysis.

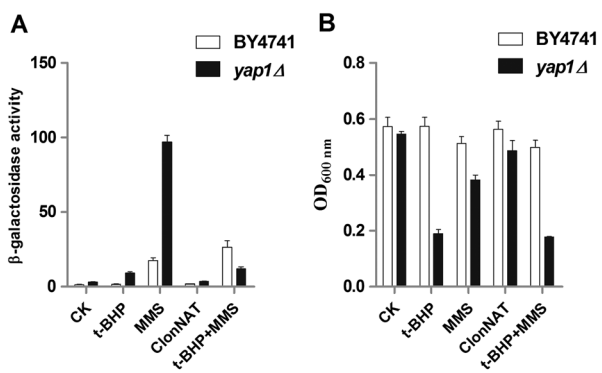

Figure 1. Responses of BY4741 (wild-type) and the *yap1* mutant to DNA-damaging agents. (A) The *RNR3–lacZ* assay. (B) Cell growth as measured by optical density (OD) at 600 nm: CK represents the untreated group; drug concentrations and exposure times were as described in the text; the results are averages of at least three independent experiments with SD

Overview of the RNA-seq data

High-throughput sequencing generated 7.17–11.67 million raw reads from each of the samples. After removing reads containing adapter, ploy-N and other low-quality reads, approximately 99% clean reads were obtained for each sample, which were mapped to the reference genome using TopHat. The percentage of total mapped reads for different samples was around 94.4–96%. In total mapped reads, the reads mapped to multiple sites accounted for 4.7–9.4%. The ratios of uniquely mapped reads used to calculate gene expression levels were 85–91%. Likewise, the percentages of non-splice reads and splice reads were 84.8–90.4% and 0.43–1.3%, respectively (see supporting information, Table S1). The read counts located in the exon region were used to estimate the gene expression level. In order to facilitate the comparison of gene expression between different samples, the gene expression level was normalized as RPKM mapped reads. As shown in Figure 2, all 10 samples displayed a similar RPKM distribution, with the median range 41.6–50.2.

Validation of RNA-seq data using qPCR

Twenty-four genes related to stress responses were selected from each comparison group for a qPCR confirmation, along with the primer sequences displayed in Table S2 (see supporting information). The expression data of genes measured by RNA-seq and qPCR are shown in Table S3 (see supporting information). The data from RNA-seq were generally confirmed by qPCR. As shown in Figure 3, linear regression analysis demonstrated an excellent

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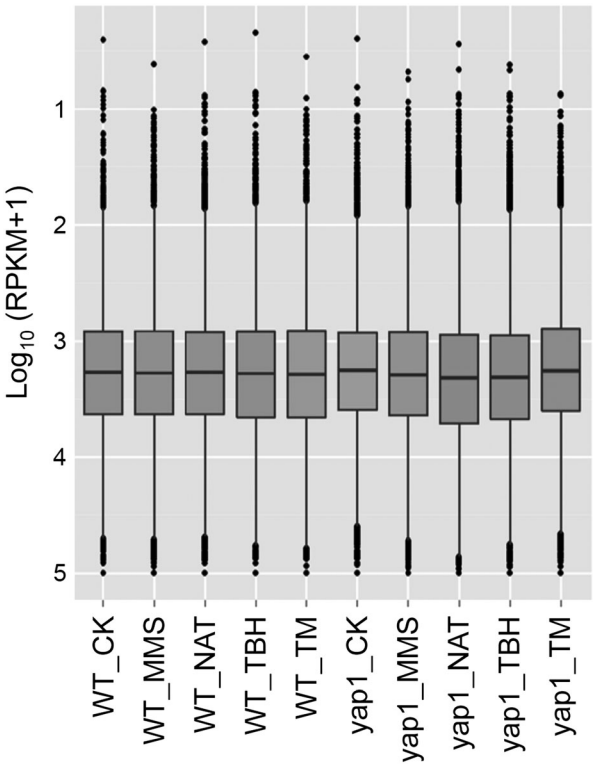

Figure 2. RPKM distribution for each sample: TM, *t*-BHP plus MMS exposure group. In each sample, the statistics from top to bottom are maximum, third quartile, median, first quartile and minimum

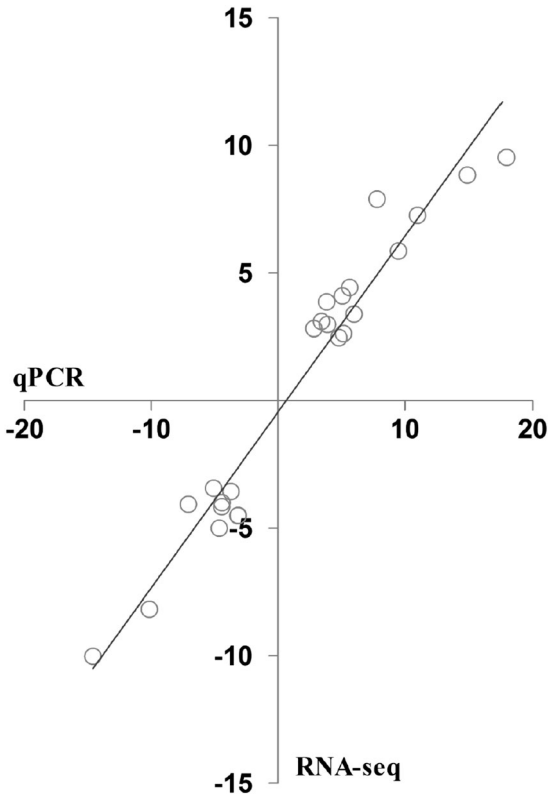

Figure 3. Correlation between qRT–PCR and RNA-seq data. Fold changes of gene expression detected by RNA-seq were plotted against the results of qRT–PCR (qPCR); the reference line indicates the linear relationship between the two methods

correlation between the two methods on both up- and downregulated genes, with $R^2 = 0.9477$.

Cluster analysis of differentially expressed genes from each comparison group

The differentially expressed genes (DEGs) between wild-type and the *yap1* mutant were analysed under both untreated and treated conditions. Genes with adjusted p value < 0.005 and $\log_2$ (fold change) > 1 were considered to be differentially expressed.

As shown in Figure 4, hierarchical clustering was analysed with DEGs from each comparison group. Cluster analysis was used to determine differences in gene expression patterns under different experimental conditions. Samples yap1_CK, WT_CK, WT_NAT and WT_TBH shared similar expression patterns. This indicated that *yap1* mutation does not cause significant changes of expression pattern with wild-type strain. After exposure to ClonNAT and *t*-BHP, the expression pattern in the wild-type does not change a lot between CK groups, indicating that *t*-BHP and ClonNAT caused minimal impact on wild-type cells. Samples yap1_MMS, WT_MMS and WT_TM also shared similar expression patterns, indicating that MMS exposure caused similar effects on *yap1* mutant and wild-type cells. Moreover, samples yap1_TM, yap1_NAT and yap1_TBH shared similar expression pattern, compare with the expression pattern of wild-type cells, indicating that ClonNAT and *t*-BHP exposure could cause significant changes in the *yap1* mutant.

Analysis of specific DEGs between wild-type and the *yap1* mutant

All of the DEGs between each comparison group are listed in List S1 (see supporting information). With untreated cells, 115 genes were differentially

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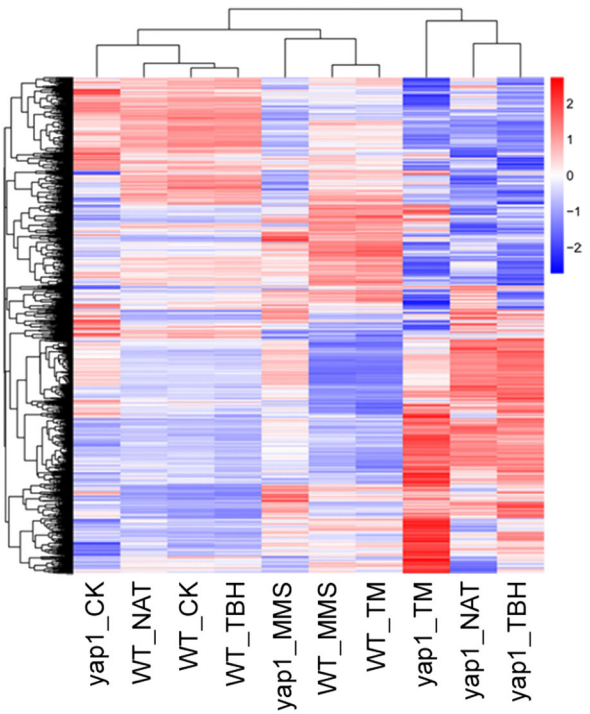

Figure 4. Cluster of differentially expressed genes. Treatment groups are indicated at the bottom; TM, *t*-BHP plus MMS exposure group. Clustering with $\log_{10}$ (RPKM + 1); red, high-expression genes; blue, low-expression genes

expressed in the *yap1* mutant compared with wild-type strain (yap1_CK vs WT_CK). Among these DEGs, 40 were upregulated and 75 downregulated. Several stress response genes, such as *WSC4* (endoplasmic reticulum membrane protein), *HTA2* (histone H2A), *HTB2* (histone H2B), *PDR12* (plasma ABC transporter protein) and *ANB1* (translation elongation factor eIF-5A), were upregulated in the *yap1* mutant, indicating that cells become sensitive to environmental stress when *YAP1* is inactivated. Meanwhile, genes related to cell fusion, oxidative stress response and mitochondrial function, such as *FUS1* (membrane protein localized to the shmoo tip), *MFA1* (mating pheromone α-factor), *OYE3* (conserved NADPH oxidoreductase), *GCV1* (T/H subunit of the mitochondrial glycine decarboxylase complex) and *PRR2* (serine/threonine protein kinase), were downregulated in the *yap1* mutant.

ClonNAT is produced by the soil bacterium *Streptomyces noursei* and belongs to a subgroup of antibiotics (Hentges *et al.,* 2005). It was used as a non-genotoxic control agent in our previous study and indeed DNA damage sensors do not respond to the ClonNAT treatment, regardless of wild-type or mutant cells (Wei *et al.,* 2013). In this study, after exposure to 20 μg/ml ClonNAT, 612 genes were differentially expressed between the *yap1* mutant and wild-type strains (yap1_NAT vs WT_NAT), among which 82 DEGs were found in both yap1_NAT vs WT_NAT and the control groups (yap1_CK vs WT_CK). The remaining 530 specific DEGs were obtained in the yap1_NAT vs WT_NAT group, in which 404 were upregulated and 126 downregulated. Most of the upregulated genes were related to rRNA synthesis, processing and assembly. Genes such as *GIP1* (meiosis-specific regulatory subunit), *FIG2* (cell wall adhesin), *STE2* (receptor for α-factor pheromone), *PMA2* (plasma membrane H^+-ATPase), *EEB1* (involved in lipid metabolism and detoxification), *WSC3* (maintenance of cell wall integrity) and *ATR1* (multidrug efflux pump) were severely downregulated, indicating that functions related to meiosis, detoxification, cell wall integrity and efflux pump were affected by *YAP1* deletion. We infer that, after exposure to ClonNAT, the *yap1* mutant exhibits stronger synthesis activity and become more sensitive to xenobiotics than wild-type cells.

In the 100 ppm MMS exposure group, 303 DEGs were found between *yap1* and wild-type strains (yap1_MMS vs WT_MMS), among which 43 yap1_MMS vs WT_MMS specific DEGs (Figure 5A) contained 23 upregulated genes, including *TIR1*, *TIR2*, *TIR3*, *TIR4* (cell wall mannoprotein of the Srp1/Tip1 family of serine–alanine-rich proteins), *DAN1* (cell wall mannoprotein with similarity to Tir1, Tir2, Tir3 and Tir4), *HUG1* (protein involved in the Mec1-mediated checkpoint pathway that responds to DNA damage or replication arrest), *CIN5* [basic leucine zipper (bZIP) transcription factor of the yAP-1 family], *RNR3*, *RNR4* (ribonucleotide-diphosphate reductase) and many components of ribosomal subunits. Since genes related to stress response and DNA damage response were upregulated after MMS exposure in the *yap1* mutant, the *YAP1* gene must play an important role in protecting cells from DNA damage. On the other hand, *TRX2*, *GSH1*, *SRX1*, *TSA1* and *NAR1*, involved in resistance to oxidative stress, were among 20 downregulated genes in the *yap1* mutant, consistent with previous reports that the Yap1 transcription factor serves as an ROS sensor for the oxidative

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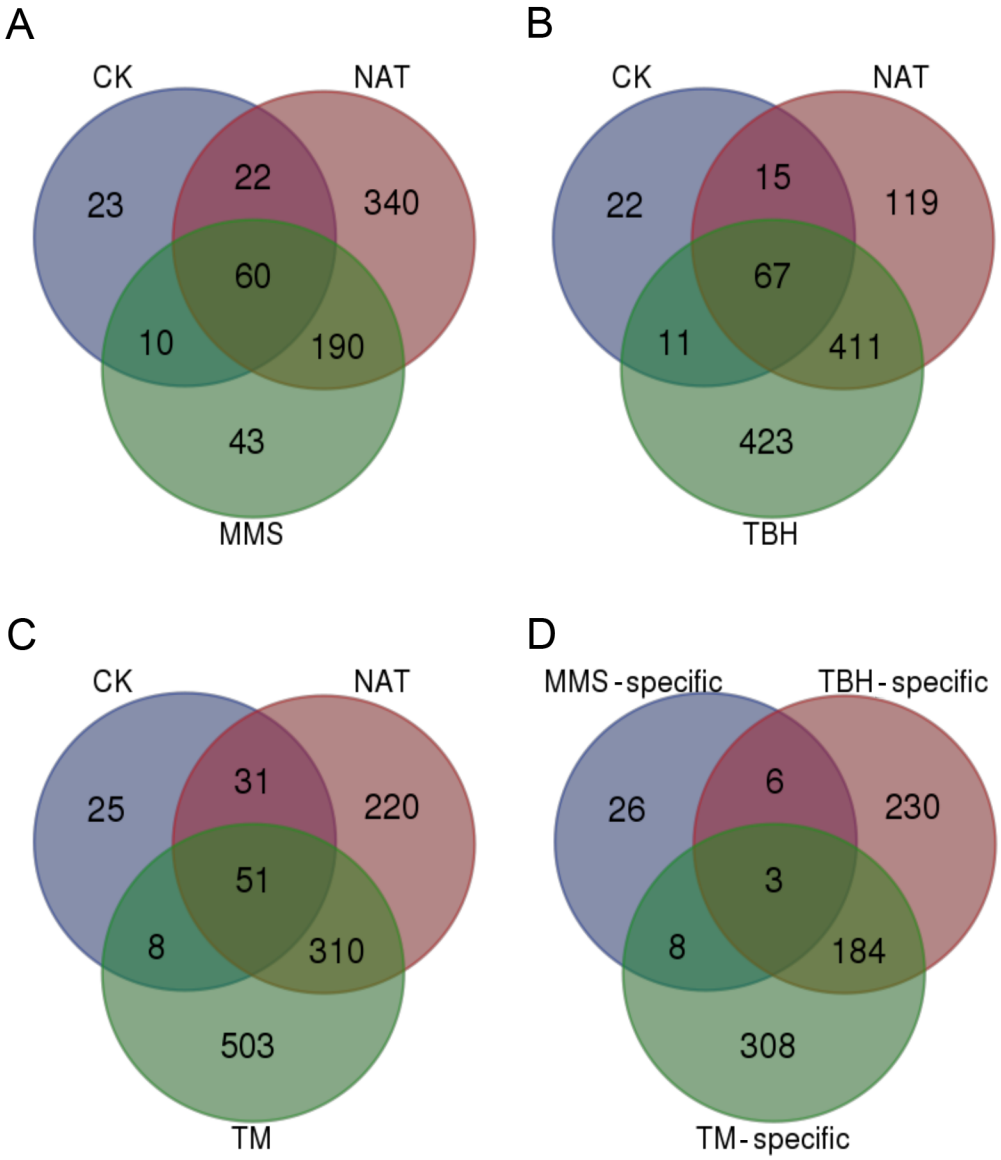

Figure 5. Venn diagram of genes in different exposure groups: (A) DEGs comparison of MMS exposure group with CK and ClonNAT exposure group; (B) DEGs comparison of *t*-BHP exposure group with CK and ClonNAT exposure group; (C) DEGs comparison of MMS and *t*-BHP mixture exposure group with CK and ClonNAT exposure group; (D) specific DEGs comparison of MMS plus *t*-BHP mixture exposure group with MMS single and *t*-BHP single exposure groups

stress response (Coleman *et al.,* 1999). Genes related to iron transport and DNA repair, such as *FTR1*, *FET3*, *RAD59* and *RAD28*, were also downregulated in *yap1* cells after MMS exposure, indicating that MMS also causes oxidative stress and genes related to anti-oxidation could not be induced due to lack of oxidative stress response, which makes the *yap1* mutant more sensitive to compounds that generate oxidative damage.

After exposure to 0.1 mM *t*-BHP, a model oxidative agent, 912 genes were differentially expressed in *yap1* mutant compared with wild-type cells (yap1_TBH vs WT_TBH), with 423 specific in the yap1_TBH vs WT_TBH group (Figure 5B), in which 181 DEGs were upregulated and 242 downregulated. In addition to DEGs common to the ClonNAT exposure, DEGs unique to the *t*-BHP exposure include *HUG1*, *CDC45*, *RAD51*,

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OLA1, *URM1* and *AQR1*, which are related to the DNA damage response, DNA replication, recombination repair, oxidative stress response and multidrug transporter, were highly upregulated in the *yap1* mutant. We infer that after exposure to *t*-BHP, oxidative stress leads to severe DNA damage in *yap1* cells. Most downregulated DEGs are related to heat shock protein, mitochondrial functions and arginine synthesis, indicating that the stress response capacity is compromised in *yap1* cells.

In a natural environment, toxicants are often present as a mixture. To verify whether the mixed exposure differs from exposure separately, 0.1 mM *t*-BHP and 100 ppm MMS were mixed for the treatment. In this treatment group, 872 DEGs between wild-type and the *yap1* mutant (yap1_TM vs WT_TM) were identified. After excluding DEGs overlapped with yap1_CK vs WT_CK and yap1_NAT vs WT_NAT groups, 503 genes were obtained (Figure 5C). In order to highlight the characteristics of mixed exposure, overlapped DEGs with *t*-BHP or MMS single exposure group were also excluded. Finally, as shown in Figure 5D, 308 specific DEGs were obtained in the *t*-BHP and MMS mixture exposure (yap1_TM vs By_TM) group, in which 137 were upregulated and 171 downregulated. Genes related to nucleolar protein, rRNA biogenesis, processing and repair of mitochondrial DNA damage were highly upregulated in this comparison group, whereas in the downregulated subsets genes related to oxidative stress response, TCA cycle and mitochondrial function were highly downregulated. We conclude that the toxic effects caused by the mixture were more severe than the two chemicals treated separately, possibly because of the vigorous rRNA synthesis. Moreover, from the downregulated DEGs in this comparison group, we found that aerobic respiration and energy generation pathways were affected by *YAP1* deletion.

Gene ontology (GO) and KEGG pathway enrichment analysis of DEGs between wild-type and the *yap1* mutant

GO enrichment analysis was performed to reveal biological processes over-represented in *yap1* mutant compared with wild-type under different chemical exposure conditions. Highly enriched or specific GO terms are shown in Figure 6. All significantly enriched GO terms are listed in List S2 (see supporting information). The KEGG pathway enrichment was analysed with the DEGs between wild-type and the *yap1* mutant under different exposure conditions representing our knowledge on the molecular interaction and reaction networks. Parts of the enriched pathways are shown in Figure 7. All of the significantly enriched KEGG pathways are listed in List S3 (see supporting information).

In the untreated group (yap1_CK vs WT_CK) there were no enriched GO terms and only the ribosome pathway was enriched in upregulated DEGs. In downregulated DEGs, 120 GO terms, such as response to chemical, conjugation, protein folding, cellular amino acid metabolic process and transcription from RNA polymerase II promoter, were significantly enriched and 10 pathways, including protein processing in endoplasmic reticulum, MAPK signalling, endocytosis and some amino acid metabolism pathways, were also significantly enriched. This indicates that deletion of *YAP1* could affect diverse pathways, especially in some regulation and stress-response processes. Thus, we conclude that the stress-response capacity has been reduced in the *yap1* mutant, making the *yap1* mutant more sensitive to chemical exposure.

After excluding overlapped DEGs with CK group, 220 GO terms were significantly enriched in the upregulated ClonNAT exposure group (yap1_NAT vs WT_NAT). The main terms included cytoplasmic translation, rRNA processing, ribosomal small subunit biogenesis, ribosomal large subunit biogenesis and nuclear transport, in which 85.6% upregulated DEGs belonged to cellular metabolic process. Of 11 pathways enriched in the upregulated DEGs in the *yap1* mutant in comparison to wild-type, ribosome, RNA transport, RNA polymerase and ribosome biogenesis pathways were highly enriched. As ClonNAT does not cause DNA damage in yeast cells, GO and KEGG pathway enrichment analysis of the upregulated DEGs revealed that the *yap1* mutation mainly enhances metabolic processes to respond to general chemical pressure. The GO enrichment analysis for downregulated DEGs showed that 12 GO terms, such as response to chemicals, α-amino acid metabolic process, multi-organism process, small molecule biosynthetic process and reproductive process in single-celled organism, were significantly enriched. Moreover, eight pathways, including butanoate, sulphur, glutathione metabolisms, MAPK signalling

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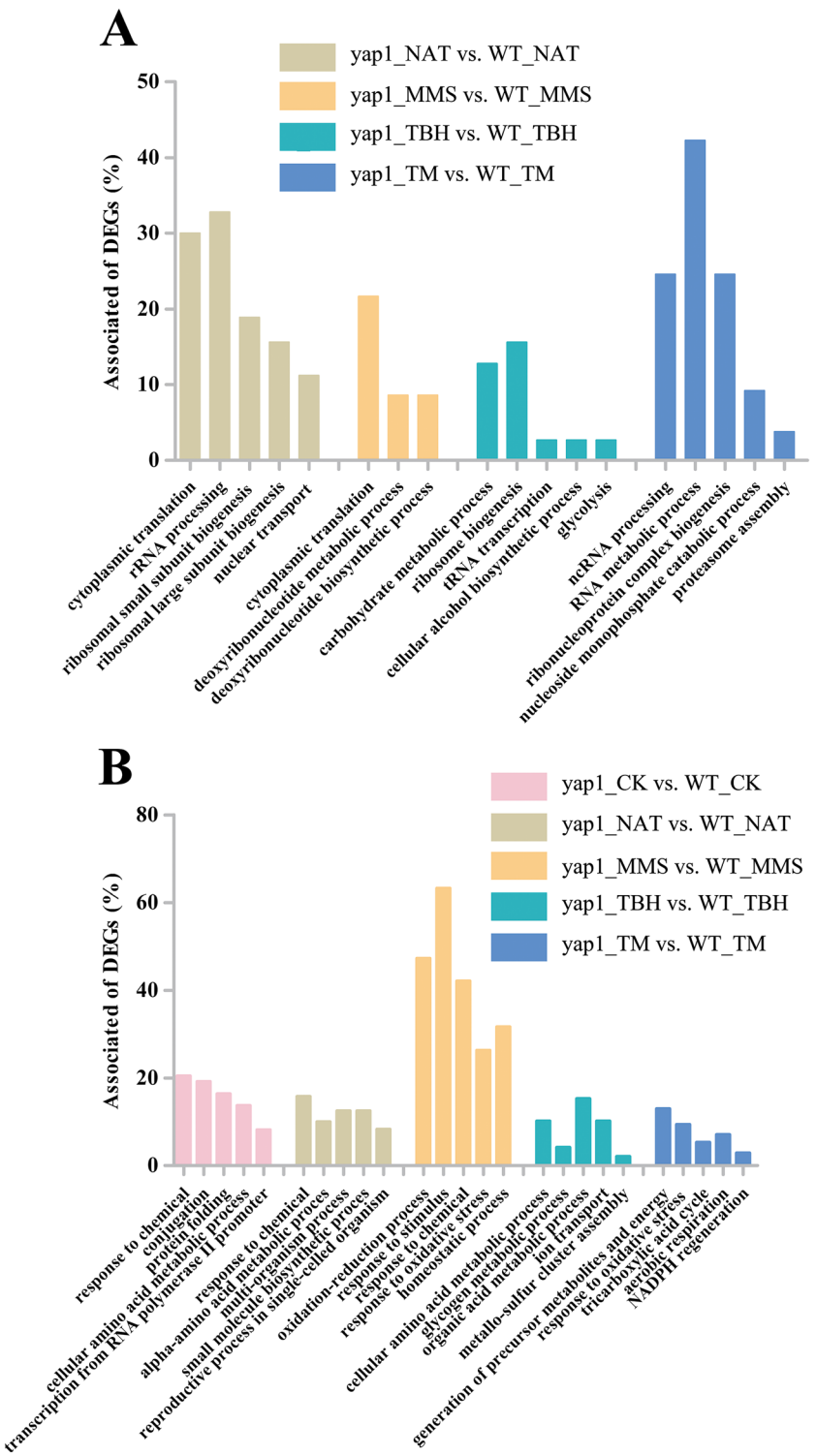

Figure 6. Highly enriched GO terms with varied percentages of DEGs in each comparison group: (A) upregulated GO terms in each comparison group; (B) downregulated GO terms in each comparison group; *y* axis represents the percentages (%) of GO term-associated DEGs in total DEGs

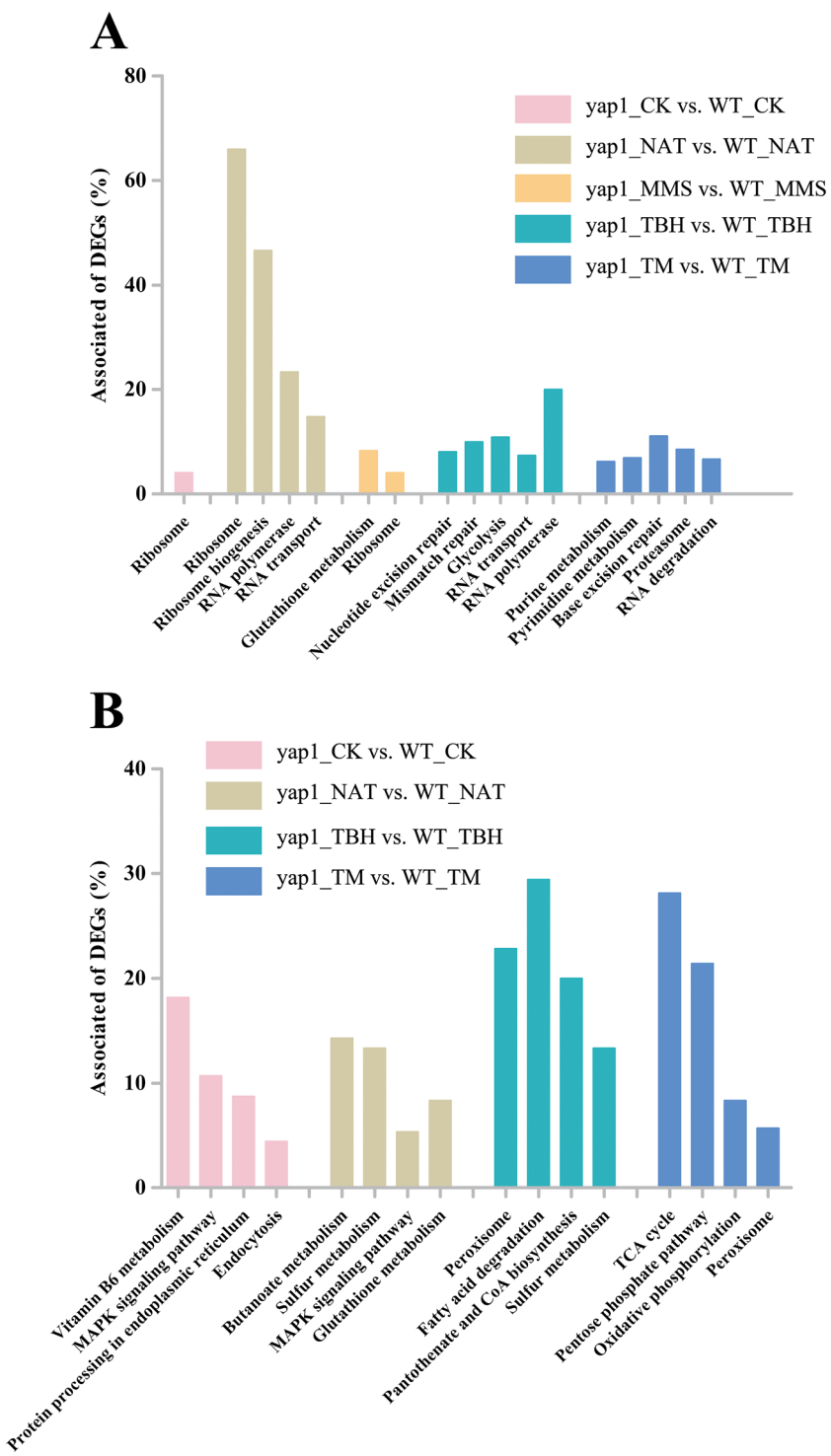

Figure 7. Highly enriched KEGG pathways with varied percentages of DEGs in each comparison group: (A) upregulated KEGG pathways in each comparison group; (B) downregulated KEGG pathways in each comparison group; *y* axis represents the percentages (%) of KEGG pathway-associated DEGs in total DEGs

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and some amino acid metabolism pathways, were significantly enriched in the downregulated DEGs. These results indicate that multiple biological processes were affected by *YAP1* deletion.

In the 100 ppm MMS exposure group (yap1_MMS vs WT_MMS), three GO terms, including deoxyribonucleotide metabolic process, deoxyribonucleotide biosynthetic process and cytoplasmic translation, were significantly enriched, while ribosome and glutathione metabolism pathways were significantly enriched in the *yap1* mutant (excluding overlapped DEGs with the CK and ClonNAT exposure groups). In the downregulated DEGs of this comparison group, 31 GO terms, including oxidation–reduction process, response to stimulus, response to chemical, response to oxidative stress and homeostatic process, were significantly enriched. No pathways were enriched while GO terms such as response to oxidative stress and oxidation–reduction process were downregulated in the *yap1* mutant, indicating that MMS also causes oxidative damage.

In the 0.1 mM *t*-BHP exposure group (yap1_TBH vs WT_TBH), the transcriptional profiles of *yap1* mutant and wild-type were completely different. Seventeen GO terms were significantly enriched by the specific upregulated DEGs (excluding the overlapped DEGs with CK group and ClonNAT exposure group). Similar to the ClonNAT exposure group, most of the GO terms were related to synthesis and metabolism processes, such as the carbohydrate metabolic process, ribosome biogenesis and tRNA transcription. However, GO terms such as glycolysis and cellular alcohol biosynthetic process specific in the *t*-BHP group were significantly enriched. It is inferred that, due to acute oxidative stress, these special pathways become prevalent in the *yap1* mutant after *t*-BHP exposure. Meanwhile, a total of 16 pathways were over-represented in the specific upregulated DEGs, such as RNA transport, RNA polymerase, RNA degradation, glycolysis, mismatch repair and nucleotide excision repair. Among downregulated DEGs, 45 GO terms, including cellular amino acid metabolic process, glycogen metabolic process, organic acid metabolic process, ion transport and metallo-sulphur cluster assembly, and 23 pathways, including peroxisome, degradation of macromolecules and some amino acid metabolism pathways, were significantly enriched. The affected pathways under acute oxidative stress in the *yap1* mutant suggest that *YAP1* plays a central role in the regulation of cellular response to oxidative damage in yeast cells.

In the mixture of 0.1 mM *t*-BHP and 100 ppm MMS exposure group (yap1_TM vs WT_TM), 99 GO terms, including ncRNA processing, RNA metabolic process, ribonucleoprotein complex biogenesis, nucleoside monophosphate catabolic process and proteasome assembly, and 11 pathways, such as purine and pyrimidine metabolism, base excision repair, RNA degradation and proteasome, were highly enriched in the specific upregulated DEGs in *yap1* mutant in comparison to wild-type cells. More genes involved in macromolecule metabolic terms were upregulated in the *yap1* mutant upon mixed exposure than either single exposure, indicating that damage caused by the mixture was greater than the single chemical exposure. For specific downregulated DEGs, 72 GO terms, including generation of precursor metabolites and energy, response to oxidative stress, tricarboxylic acid cycle, aerobic respiration and NADPH regeneration, and a total of 19 pathways, including TCA cycle, oxidative phosphorylation, peroxisome, glutathione metabolism and some amino acid metabolism, were significantly enriched. Multiple pathways were affected in the *yap1* mutant upon exposure to mixed toxins, especially the downregulation of aerobic respiration and related pathways, suggesting that mitochondrial functions are severely damaged by the mixture of *t*-BHP and MMS.

Discussion

YAP1 encodes an AP-1 type transcription factor and is a key player in the cellular response to oxidative stress, xenobiotic insults and heavy metal stress (Coleman *et al*., 1999; Delaunay *et al.,* 2000; Rodrigues-Pousada *et al.,* 2004). Our transcriptomic profiles showed that, under non-exposure conditions, there are 115 DEGs in the *yap1* mutant. Most effects of *yap1* mutation are related to reproduction processes, suggesting that the *yap1* mutant is vulnerable to the environment. Because of a powerful repair system in the wild-type cells, the DEGs to some GO terms related to stress response, such as DNA damage response or oxidative stress response, were not significantly enriched after genotoxic agent exposure. However, the DEGs to those GO terms are highly enriched in the *yap1* mutant under the

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exposure condition, indicating that *YAP1* deletion causes an accumulation of damage in the cell and becomes more sensitive to chemical exposure. This observation shows that the cellular response to chemical exposure is not only dependent on the chemical type but also on the genetic variability of the cells. Based on this, we can establish an interaction of gene mutation and chemical exposure that enables comprehensive queries over the effects of both the genes and the chemicals. The toxicogenomics data of the interaction effect of mutant and chemicals generated by the yeast model will be a enrichment for the database of environmental risk assessment.

In general, under chemical exposure, most downregulated DEGs in the *yap1* mutant are positively regulated by *YAP1* and reflect the *YAP1* function, while most upregulated DEGs in the *yap1* mutant are responding to the accumulation of damage that is either negatively regulated by *YAP1* or unrelated to *YAP1* regulation. In this study, the yeast *yap1* mutant was compared with wild-type cells for the response to genotoxic agents, which reveals completely different transcriptomic profiles in addition to the overlapping DEGs. In this study, the transcriptomics analysis allows us to interrogate the effects of *yap1* mutation on the response to chemical exposure. Through comparison between wild-type and the *yap1* mutant cells under chemicals exposure conditions, we generated transcriptomic profiles as a chemical-specific exposure signature in susceptible individuals. The potential application of this transcriptomic profiles includes several aspects. The gene expression pattern can be used as a signature for screening of chemicals in an environmental sample. Chemicals with similar patterns of gene expression may be grouped into category, and expression patterns of an unknown chemical can be compared with the signature of well-characterized chemicals to initially assess the risk (Nuwaysir *et al.,* 1999). Furthermore, it is necessary to use susceptible individual to develop insights to fill the gap of individual sensitivity in chemical exposure risk assessment. Our results showed that the susceptible individuals were more sensitive than wide-type individuals in lower concentration exposure, because of some stress response pathways deficiency. For example, some redox homeostasis, DNA damage response and other macromolecule damage response pathways were significantly affected in the *yap1* mutant. With the accumulation of these data, one can build a correlation among chemical exposure, effective pathways and susceptible individuals (Tennant, 2002). Most importantly, because of cross-species similarity, particularly within eukaryotes, from yeast to humans, toxicogenomics data from budding yeast could be used to extrapolate human disease outcomes. To link the identified DEGs in the susceptible individual to the risk assessment, the human homologues of these DEGs and their associated disease phenotypes were searched in the database with YeastMine tool and OMIM Disease Phenotype database, from which (see supporting information, Table S4) many human homologues of these DEGs, such as *CHEK2*, *GATA2*, *AXIN1*, *KLF6*, *ELAC2*, *PANK2* and *BLMH*, were linked to cancer, ageing and neuronal degeneration when abnormally expressed or mutated. These observations suggest that some human susceptible individuals, like the yeast *yap1* mutant, bear higher chemical exposure risk than normal individuals, that these groups are more susceptible to certain diseases and that personalized clinical care is needed.

In conclusion, our results confirm previous reports that the yeast *yap1* mutant is sensitive to a broad range of stresses, because of the deficiency of the important regulation process in oxidative stress response and DNA damage response. Several toxicological effects were identified in *yap1* mutant under chemical exposure conditions, indicating that the susceptible individuals bear a higher risk of chemical exposure than normal individuals. Furthermore, these transcriptomic profiles that combined the mode of action of chemicals and individual susceptibility can be used to construct a database and as a basis of a system biology tool for environmental risk assessment. However, the 'omics' technologies used in environmental risk assessment are still at the stage of data collection and validation. More data from transcriptomic, proteomic and metabolomic studies need to be integrated to enable comprehensive understanding of the potential impacts of chemicals on human health and the environment.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1. Statistics for reads filtering and mapping (numbers and percents).

Table S2. Primer sequences for validated genes.

Table S3. Comparisons between RNA-seq data and qPCR results.

Table S4. Human orthologues of DEGs and related diseases.

List S1. DEGs in each comparison group.

List S2. Significantly enriched GO terms in each comparison group.

List S3. KEGG pathway enrichment analysis for each comparison group.

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