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Applying Genome-wide CRISPR to Identify Known and Novel Genes and Pathways that Modulate Formaldehyde Toxicity

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Abstract

Formaldehyde (FA), a ubiquitous environmental pollutant, is classified as a Group I human carcinogen by the International Agency for Research on Cancer. Previously, we reported that FA induced hematotoxicity and chromosomal aneuploidy in exposed workers and toxicity in bone marrow and hematopoietic stem cells of experimental animals. Using functional toxicogenomic profiling in yeast, we identified genes and cellular processes modulating eukaryotic FA cytotoxicity. Although we validated some of these findings in yeast, many specific genes, pathways and mechanisms of action of FA in human cells are not known. In the current study, we applied genome-wide, loss-of-function CRISPR screening to identify modulators of FA toxicity in the human hematopoietic K562 cell line. We assessed the cellular genetic determinants of susceptibility and resistance to FA at 40, 100 and 150 μM (IC10, IC20 and IC60, respectively) at

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Author Contributions

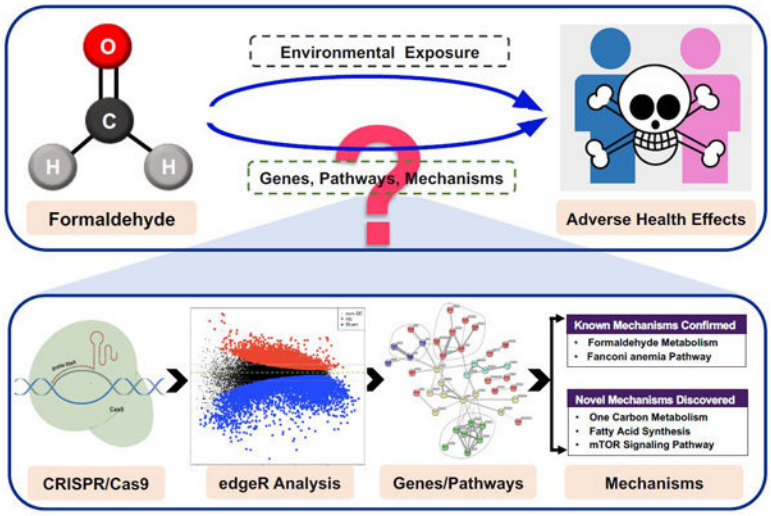
L.Z., C.D.V conceived the original project. Y.Z. led the project and designed and performed the majority of experiments. A.T. and A.S. conducted CRISPR library construction experiments. L.W. and A.L. conducted the bioinformatic analysis. C.J.C. provided expertise and feedback. Y.Z. drafted the manuscript with the guidance from C.M.C., C.D.V. and L.Z.

Declaration of Interests

The authors declare no competing financial interest.

two time points, day 8 and day 20. We identified multiple candidate genes that increase sensitivity (e.g. *ADH5*, *ESD* and *FANC* family) or resistance (e.g. *FASN* and *KMD6A*) to FA when disrupted. Pathway analysis revealed a major role for the FA metabolism and Fanconi anemia pathway in FA tolerance, consistent with findings from previous studies. More network analyses revealed potential new roles for one-carbon metabolism, fatty acid synthesis and mTOR signaling in modulating FA toxicity. Validation of these novel findings will further enhance our understanding of FA toxicity in human cells. Our findings support the utility of CRISPR-based functional genomics screening of environmental chemicals.

Graphical Abstract



Keywords

Formaldehyde; CRISPR Screen; Formaldehyde Metabolism; DNA Repair; Fanconi Anemia

1. Introduction

Formaldehyde (FA), the simplest aldehyde and widely used industrial chemical is utilized in construction, in the manufacture of furniture and textiles and in embalming fluid as a tissue preservative (Duong et al., 2011; Zhang et al., 2010). Exposure to FA has been linked to a range of adverse health effects (Tang et al., 2009). Moreover, FA is classified as a Group I human carcinogen by International Agency for Research on Cancer (IARC) as it causes nasopharyngeal cancer and is associated with myeloid leukemia (IARC 2012).

Previously, we reported that FA induced hematotoxicity and chromosomal aneuploidy in exposed workers (Lan et al., 2015; Tang et al., 2009; Zhang et al., 2010), toxicity in bone marrow and hematopoietic stem/progenitor cells (HSC/HPCs) of exposed mice (Wei et al., 2017; Ye et al., 2013), and other toxic effects in human lymphoblastoid cell lines (Ren et al., 2013). FA is a genotoxicant that predominantly induces DNA-protein crosslinks (DPC) whose partial repair results in permanent DNA alterations (Wong et al., 2012; Quievryn and Zhitkovich, 2000; Barker et al., 2005). Additionally, FA is a potent proteotoxic agent that

may induce extensive protein damage such as accumulation of abnormal proteins and DNA damage-independent cytotoxicity (Ortega-Atienza et al., 2016). Despite these, mechanistic information is still limited; and, the molecular and cellular processes underlying the toxicity induced by FA require additional research.

Functional toxicogenomic profiling, a powerful tool to study molecular and cellular toxicity and genetic susceptibility, has been used to test FA in various models (McHale et al., 2014; North and Vulpe, 2010). Using the chicken DT40 model (Yamazoe et al., 2004), deficiencies in the Fanconi anemia/breast cancer-associated (FANC/BRCA) pathway and homologous recombination (HR) were shown to induce hypersensitivity to FA (Ridpath et al., 2007). In *Saccharomyces cerevisiae*, genome-wide functional screening revealed potential mechanisms of FA sensitivity and tolerance (North et al., 2016). Furthermore, by studying mutants of *KBM7*, a human chronic myeloid leukemia cell line with near-haploid haploid DNA content, we identified several resistant genes that modulate susceptibility to FA (Shen et al., 2016). While informative, these model systems and approaches do not fully recapitulate the factors and processes mediating FA toxicity in diploid human cells. Therefore, in the current study, we applied clustered regularly interspaced short palindromic repeats (CRISPR) based screening approaches to identify potential mechanisms and biological pathways involved in FA toxicity in the human K562 cell line.

CRISPR-associated protein 9 (Cas9), a powerful gene editing tool, has enabled genome-wide screening in mammalian cells to uncover molecular modulators and mechanisms of biological processes (Hsu et al., 2014; Kampmann, 2018; Shalem et al., 2014). CRISPR-Cas9 loss-of function screening has been used to identify genes and pathways modulating susceptibility to chemotherapeutics and biological toxins and some environmental toxicants and chemicals (Sobh and Vulpe, 2019). Using genome-wide CRISPR screening, we recently identified potential mechanisms of cellular toxicity induced by arsenic trioxide (Sobh et al., 2019b) and acetaldehyde (Sobh et al., 2019a) in human leukemia K562 cells, while others studied the arsenic-induced endoplasmic stress and apoptosis (Panganiban et al., 2019) and the toxicity induced by triclosan (Xia et al., 2016), paraquat (Reczek et al., 2017), and benzo[a]pyrene (Sundberg and Hankinson, 2019). In the current study, we performed a loss-of-function genome-wide CRISPR screen in human K562 cells exposed to FA to identify relevant genes and pathways that modulate FA cytotoxicity.

2. Materials and Methods

2.1 Cell cultures

Human HEK293T (for lentivirus production) and K562 cells (for CRISPR screen) were obtained from the Cell Culture Facility, Biosciences Divisional Services, University of California, Berkeley. The HEK293T cells were cultured in Dulbecco's Modified Eagles Media (DMEM; Thermo Fisher Scientific, Waltham, MA) and the K562 cells were cultured in RPMI 1640 media (Thermo Fisher Scientific, Waltham, MA). Both types of medium were supplemented with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA) and 1% penicillin-streptomycin. Cells were cultured in a humidified incubator with 5% CO_2 at 37°C.

2.2 FA treatment and cytotoxicity assay

Two rounds of cell viability tests were conducted by measuring cellular ATP level using the CellTiter-Glo luminescent cell viability assay kit (Promega, Madison, WI) according to the manufacturer's protocol. Briefly, human K562 cells were seeded into opaque 96-well cell culture plates at a density of 1×10^5 cells/ml (10^4 cells/well). Cells were treated with 0–625 μM FA (Thermo Fisher Scientific, Waltham, MA) (1st experiment) and 0–150 μM FA (2nd experiment), respectively, for 72 h (Figure 1A, 1B). At the end of treatment, the cells in each well were mixed with same amount (100 μl) of CellTiter-Glo reagent and the cell plate was incubated in the dark at room temperature. After 10 min incubation, luminescent signals were read on a Synergy H1 microplate reader (BioTek Instruments, Winooski, VT). During the CRISPR screen, different doses of FA at IC10 (40 μM), IC20 (100 μM) and IC60 (150 μM) were used to treat human K562 cells continuously for 20 days. Cells in each flask were passaged every 2 days and fresh FA was added along with the new medium. On days 8 and day 20, an aliquot of cells (30×10^6 cells) was collected from each replicate of each treatment and kept at −80 °C for future sequencing. Generally, in CRISPR-based functional toxicogenomic screening, high doses (e.g. >IC50) over relatively long exposure periods are more effective for the selection of resistant clones, whereas low doses (e.g. IC10) for shorter exposure periods are more effective for selective of sensitive clones (Sobh and Vulpe, 2019; Xia et al., 2016).

2.3 CRISPR library

The human genome-wide CRISPR knockout pooled library (Brunello, cloned in LentiCRISPRv2 backbone) was obtained from Addgene. The Brunello library consists of 76441 single guide RNAs (sgRNAs) targeting 19114 genes with an average of 4 sgRNAs per gene and 1000 non-targeting control sgRNAs (Sanson et al., 2018). The library was amplified following a previously published protocol (Shalem et al., 2014). After the library amplification, lentiviruses were produced in HEK293T cells by co-transfection of Brunello library plasmids together with packaging plasmid psPAX2 (Addgene) and envelope plasmid pMD2.G (Addgene).

2.4 Genome-wide screening

The Lentiviral library (10 μl) and 8 μg/ml polybrene (Sigma, St. Louis, MO) were transduced into 100×10^6 K562 cells seeded in 12-well plates with 2.5×10^6 cells per well, resulting in a low multiplicity of infection (MOI), around 0.3–0.5. The plates were centrifuged at 1000 g for 2h at 34°C. After infection, all transduced cells were resuspended to remove polybrene and non-transfected virus and pooled in fresh media. After 48h recovery, puromycin (2 μg/ml) selection was performed for 5–7 days, with fresh media were supplied every 48h, to eliminate non-transduced cells. After puromycin selection, aliquots of 30×10^6 cells were transferred into T225 cell culture flasks to maintain a 400-fold representation of the 76441 sgRNAs in the Brunello library. The cells were treated in triplicate with vehicle (phosphate buffer solution, PBS) or FA at 40, 100 and 150 μM inducing inhibitory concentration IC10, IC20 and IC60, respectively, and incubated for 20 days (Figure 1C). Fresh media (including FA, as appropriate) was added to each flask every

2 days. A total of 30×10^6 cells from each replicate of each treatment on day 8 and day 20 were centrifuged and the resulting pellets were stored at −80°C for DNA extraction.

2.5 Next generation sequencing

Genomic DNA was extracted from the cell pellets archived from the genome-wide CRISPR screening using the Quick DNA plus kit (ZYMO, Irvine, CA) following the manufacturer's protocol. Herculase II Fusion DNA Polymerase kit (Agilent, Santa Clara, CA) was used to amplify the pool of sgRNA sequences/barcodes in the genomic DNA for next generation sequencing (NGS). 100 μg DNA (8.3 μg genomic DNA/reaction; 12 replicates/sample) was amplified from each sample. The PCR conditions were as follows: 95°C/3 min; 28 cycles of 95°C/30 s, 60°C/30 s, 72°C/25 s, and 72°C/2 min. To enable multiplexing of samples for sequencing, multiple reverse primers—each with a unique 8 bp barcode (Table S1)—were used in the PCR and to label each sample. All 12 PCR reactions for each sample were pooled. The quality of the PCR amplicons was assessed by gel electrophoresis in 2% agarose and analysis with a 2100 bioanalyzer (Agilent, Santa Clara, CA). If needed, Qiaquick PCR purification kits were used to remove unincorporated primers and spurious products. Bands of the expect size, 256 bp, were excised from the gel and Qiaquick gel extraction kit was used to isolate PCR products for all samples. Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA) was used to quantify individual samples labeled with different indices. All samples then were pooled in equimolar amounts and deep sequenced (280,330 million reads per lane) using the Illumina Hiseq4000 platform at QB3 facility at the University of California, Berkeley (single read 150 bp).

2.6 Bioinformatic and statistical analysis

FASTX-Toolkit was used to demultiplex raw FASTQ data files, which were further processed to generate reads containing only the unique 20 bp guide sequences. The sgRNA sequences from the library were assembled into a Burrows-Wheeler index (Li and Durbin, 2009) using Bowtie build-index function (Langmead et al., 2009). Reads were then aligned to the index using the Bowtie aligner. The efficiency of alignment was checked and the number of uniquely aligned reads for each library sequence was calculated to create a table of raw counts. The normalization method in package edgeR was applied to normalize raw counts and differentially expressed genes were determined accordingly. The heatmap of normalized sgRNA counts using edgeR is presented as Figure S1. Since edgeR uses negative binomial model to conduct normalization, it does not transform the original read counts. Using edgeR, all candidate sgRNAs with false discovery rates (FDRs) <0.01 were identified. The candidate genes were identified with at least 2 sgRNAs consistently modulating sensitivity or resistance to FA at FDR<0.01 (Figure 1D). Pathway Studio (Nikitin et al., 2003) and KEGG mapper were used to perform pathway analysis. DAVID and STRING databases were applied for the functional annotation clustering of candidate gene analysis. All of our CRISPR screening data will be deposited in the Open Repository of CRISPR Screens (ORCS) database.

GraphPad Prism version 6 was used to perform statistical analysis for FA cytotoxicity test. Data were expressed as mean ± SEM. Data were analyzed by one-way ANOVA.

3. Results

3.1 Identification of candidate genes modulating cellular susceptibility to FA using genome-wide CRISPR

We carried out a genome-wide CRISPR-Cas9 loss-of-function library screening in human K562 cells exposed to FA at three doses (40 μM, 100 μM and 150 μM, corresponding to the IC10, IC20 and IC60 respectively) in addition to a vehicle control (Figure 1A–1C) for two time points (8 and 20 days). Among all doses and time points, the most robust differential responses were observed with 100 μM and 150 μM FA at day 8. At day 20, we observed only a few differential sensitivities for 100 μM and 150 μM FA. Similarly, a very limited number of candidates were identified at 40 μM FA at either 8 or 20 days. Thus, most data for 40 μM FA and day 20 are included in supplementary information (Figure S2, S3; Table S2, S3) as illustrated in Figure 1D.

sgRNA representation in each sample was determined by NGS and candidate differentially sensitive mutants (FA-exposed as compared to vehicle controls) were determined by the edgeR algorithm (Figure 2A, 2B, Figure S2). We identified candidate genes as those for which loss of function mediated by at least 2 sgRNAs concordantly affected cellular sensitivity or resistance to FA at FDR<0.01. Based on these criteria at day 8, 497 and 538 candidate genes were associated with increased sensitivity to 100 μM and 150 μM FA, respectively (Figure 2C); and, 404 and 433 candidate genes were associated with increased resistance to 100 μM and 150 μM FA, respectively (Figure 2C).

At day 20, loss of function of *ADH5* and *ESD* conferred sensitivity to 100 μM FA (Table S3A) whereas loss of function of 13 and 10 genes conferred sensitivity and resistance 150 μM FA, respectively (Figure S3, Table 3B). In cells treated with 40 μM FA, only 1 gene (*CT47A5*) was associated with resistance (day 8) and only 1 gene (*ESD*) was associated with sensitivity (day 20) (Table S2).

3.2 Functional enrichment analysis of candidate genes

For functional enrichment analysis, we have selected common candidate genes more rigorously with at least 3 sgRNAs that mediated a significant effect at both 100 μM and 150 μM FA (day 8, Figure 3). A total of 62 common genes (Table S4, S5) were identified and their association with sensitivity/resistance are illustrated in venn diagram and heatmap (Figure 3A). Functional enrichment analysis using DAVID on these 62 genes revealed that multiple pathways and biological processes, including FA metabolic process, DNA repair and in particular the Fanconi anemia pathway, oxidation-reduction process and fatty acid biosynthesis and metabolism, regulate the cellular sensitivity or tolerance to FA (Figure 3B). Functional clustering analysis using STRING further confirmed the interconnected cellular processes involved in modulating FA toxicity including FA metabolism, DNA damage/repair and fatty acid synthesis (Figure 3C).

3.3 Disruption of FA metabolism genes increases FA sensitivity

Disruption of *ADH5*, *ESD*, *TKT* and *RPE* in 100 μM and 150 μM FA at day 8 rendered K562 cells more sensitive to cytotoxicity (Figure 4A, Table S5A), moreover, *ADH5* and

ESD were the top sensitivity genes at day 20 (Table S3). These genes were shown to cluster strongly and encode proteins involved in FA metabolic processes and carbon metabolism (Figure 4B, 4C). As two key genes encoding components of FA metabolism (Figure 4D), as expected, disruption of *ADH5* or *ESD* markedly increased the cellular sensitivity to FA (Table S3, S5A). Unexpectedly, disruption of *TKT* and *RPE* which encode proteins with a known role in the pentose phosphate pathway of carbon metabolism similarly increased sensitivity to FA (Figure 4D).

3.4 DNA damage/repair response is important in modulating cellular sensitivity to FA

Genes encoding components of DNA damage response/repair were also identified as top candidate genes whose disruption altered FA cytotoxicity (Figure 5A–5C). Among the 62 overlapping genes at day 8, 14 DNA repair related genes were identified which increase the FA sensitivity (Table S5A) and two genes, *KDM6A* and *KMT2C*, increased resistance (Table S5B). Notably, disruption of multiple *FANC* family genes, e.g, *FANCA*, *FANCB* and *FANCE*, as well as *ATM* markedly enhanced sensitivity to FA (Figure 5C, Figure S4). Additionally, a few components of DNA homologous repair (HR) pathway, e.g. *RAD50* and *XRCC3*, were identified as increasing sensitivity in our CRISPR screen (Figure 5D). Notably, we did not identify genes encoding components of the nonhomologous end joining pathway (NHEJ) pathway as modulating FA induced cytotoxicity.

3.5 Modulation of fatty acid biosynthesis processes affects FA cellular toxicity

Interestingly, disruption of six genes involved in fatty acid synthesis were strongly enriched or decreased by FA treatment at day 8 (Figure 6A, Table S5). Functional and clustering analysis showed that FA cytotoxicity was dramatically modulated via disruption of the fatty acid synthesis pathway (Figure 6A–6C). Among these genes, the knockout of *FASN*, a key component involved in type I fatty acid synthesis, increased the tolerance to FA while the deletion of type II fatty acid synthesis related genes, e.g. *MERC*, *OXSM* and *MCAT*, increased the cellular sensitivity to FA (Figure 6D).

3.6 mTOR signaling pathway is upregulated by FA treatment at Day 20

Unexpectedly, as noted above we identified far fewer differentially sensitive sgRNA targeted mutants at day 20 as compared to day 8. Only disruption of *ADH5* and *ESD* dramatically enriched the comparative cellular sensitivity to 100 μM FA at day 20 (Table S3A), while 13 candidate genes which increase sensitivity and 10 genes which enhance resistance, when targeted, were identified in 150 μM FA treated cells (Figure S3). In addition to a few genes associated with FA metabolism and DNA damage/repair pathway, some of which also were identified at day 8, we identified multiple GTP binding proteins, e.g. *RRAGA* and *RRAGC*, as well as mTOR (mammalian target of rapamycin) activators, e.g. *LAMTOR1*, *LAMTOR2* and *LAMTOR3*, were identified as top resistant genes highly increasing the tolerance of cells to 150 μM FA (Table S3B). These 5 genes shown in Figure 7A encode components which regulate the mTOR signaling pathway and macroautophagy (Figure 7B–7D).

4. Discussion

4.1 Genome-wide CRISPR screen is a valid approach to assess FA toxicity

Genome-wide CRISPR screening has been applied to profile the functional role of gene products involved in the cellular response and biological processes related to various exposures in mammalian cells. Notably, recent studies have investigated potential genes and cellular processes modulating environmental toxicants induced toxicity using genome-wide CRISPR screening, such as arsenic-induced endoplasmic reticulum stress associate apoptosis (Panganiban et al., 2019) and the toxicity induced by triclosan (Xia et al., 2016), paraquat (Reczek et al., 2017), and benzo[a]pyrene (Sundberg and Hankinson, 2019). We also previously applied genome-wide CRISPR to uncover the potential mechanisms of arsenic trioxide cellular toxicity, which was alleviated by the utilization of selenocysteine or the perturbation of its biosynthesis (Sobh et al., 2019b). Moreover, our previous genome-wide CRISPR study on acetaldehyde (the second smallest compound to FA in aldehyde family) revealed that the loss of DNA repair genes enhanced sensitivity and the disruption of *OVCA2*, an uncharacterized tumor suppressor, was shown to increase acetaldehyde sensitivity in human cells (Sobh et al., 2019a).

Previously, diverse model systems, including chicken DT40 cells, eukaryotic yeast *Saccharomyces cerevisiae* and human KBM7 cells, were employed to screen FA cytotoxicity via functional toxicogenomic profiling (North et al., 2016; Ridpath et al., 2007; Shen et al., 2016). These studies revealed pathways and mechanisms of FA toxicity and we validated some findings in human cell lines. However, limitations of these screening models and methods, such as difficulty extrapolating the findings to human diploid cells, promoted us to apply CRISPR-based functional profiling to study determinants of FA toxicity directly in human cells. Specifically, we applied genome-wide loss-of-function CRISPR screening in human K562 cells exposed to three doses of FA for 20 days and examined survival/growth at days 8 and 20. We confirmed previously known genes/pathways modulating FA toxicity and identified potential roles for several unknown genes/pathways.

4.2 Previously known genes and pathways modulating FA toxicity are confirmed by CRISPR screening

FA metabolism: In our CRISPR screen, *ADH5* and *ESD* were the only two sensitivity genes identified at both day 8 and day 20 following FA treatments at IC20 (100 μM) as well as IC60 (150 μM). Among all alcohol dehydrogenases, alcohol dehydrogenases 5 (ADH5) is expressed ubiquitously in all tissues and plays an important role in formaldehyde detoxification (Edenberg and McClintick, 2018; Ladeira et al., 2013). FA spontaneously reacts with glutathione to form S-hydroxymethylglutathione, which is oxidized to S-formylglutathione (Staab et al., 2009). Further, FGSH is hydrolyzed by S-formylglutathione hydrolase to generate formate, which then enters into one carbon cycle (Reingruber and Pontel, 2018; Schug, 2018). S-formylglutathione hydrolase, a carboxylic ester hydrolase, termed esterase D (ESD) is expressed in most human tissues (Gonzalez et al., 2006; Harms et al., 1996; Kaźmierczak et al., 2013; Potter et al., 2009). As thioesterases, S-formylglutathione hydrolases are highly conserved expressing throughout prokaryotes and eukaryotes, and function as a key enzyme mediating formaldehyde detoxification pathways

across evolutionarily divergent species (Cummins et al., 2006). By deleting *ADH5*, previous studies indicated that DNA adducts induced by FA were increased in multiple tissue types of mice and DT40 cells and cells were highly susceptible to FA treatment (Deltour et al., 1999; Pontel et al., 2015; Rosado et al., 2011). Consistent with these previous studies, our screen revealed that deletion of either *ADH5* or *ESD* enhanced the sensitivity of K562 cells to FA exposure. Presumably, the disruption of these two genes impeded FA detoxification, which made the K562 cells more susceptible to FA. Our findings confirm that the activity of ADH5 and ESD, two key players in the FA metabolic pathway, are vital for formaldehyde tolerance.

DNA damage and repair: Using the chicken DT40 screening model, Ridpath et al. demonstrated the essentiality of the FANC/BRCA pathway for eliminating DPCs, a strong biomarker of DNA damage and genotoxic effect induced by FA and other aldehydes in cells (Liu, 2006; Ridpath et al., 2007; Shaham, 2003; Shaham et al., 1996). Similarly, in our current study, we also found that multiple genes from *FANC* family largely regulated FA cytotoxicity (Figure S4). In addition, *ATM* was identified in our current screen as a significant regulator of FA-induced DNA damage. This fits with a previous finding that low levels of FA strongly activated the ATM signaling pathway in human cells, suggesting that ATM kinase is involved in FA resistance (Ortega-Atienza et al., 2016). HR pathway, but not the NHEJ pathway, is involved in the repair of FA-induced DNA damage (Ridpath et al., 2007, North et al., 2016). Our previous study using yeast identified several DNA homologous repair related genes, e.g. *RAD51* (human: *RAD51*) and *RAD57* (human: *XRCC3*), whose loss induced hypersensitivity to FA (North et al., 2016). Besides, using yeast, de Graaf et al. demonstrated that homologous recombination protects against chronic FA exposure (de Graaf et al., 2009). Interestingly, in the present CRISPR screen, we also identified a few genes involved in HR pathway, such as *BRCA1*, *BRCA2*, *RAD51B* and *XRCC3*, as sensitive genes at lower stringency (FDR<0.1) (Table S6), showing that the HR pathway may play a role in modulating FA induced genotoxicity. In summary, our approach identified and confirmed multiple DNA repair components whose deletion enhances cellular sensitivity to formaldehyde.

4.3 CRISPR screening reveals novel genes/pathways involved in the modulation of FA toxicity

One carbon metabolism: Loss of function of *TKT* and *PRE*, two genes encoding key enzymes involved in one carbon metabolism—previously not known to play a ro le FA toxicity—conferred increased sensitivity to F A in our CRISPR screen. In a wide range of methylotrophic bacteria, the ribulose monophosphate (RuMP) pathway involves FA fixation to the pentose phosphate pathway (PPP) intermediate ribulose-5-phosphate (Ru5P) (Bennett et al., 2018; Mitsui et al., 2000; Whitaker et al., 2015). Briefly, 3-hexulose-6-phosphate synthase (HSP) catalyzes the addition of FA to Ru5P to form D-arabino-3-hexulose 6-phosphate (H6P), which is further catalyzed to fructose-6-phosphate (F6P) upon isomerization (Figure 4D). Subsequently, F6P is transformed to xylulose-5-P (X5P) by transketolase (TKT), which undergoes epimerization by ribulose-5-phosphate-3-epimerase (RPE) to regenerate Ru5P, the acceptor of FA (Figure 4D). The process of FA fixation to F6P is only found in archaea and yeast, but not, so far, in human. The enhanced sensitivity to

FA by the disruption of *TKT* or *RPE* in our CRISPR screen suggests that other processes could potentially be present in human cells similar to the non-human FA fixation process catalyzed by HSP. In addition, dihydroxyacetone synthase (DHAS), also known as formaldehyde transketolase (ec 2.2.1.3), is found in methylotrophic yeast species catalyzing X5P to yield glycerone via FA fixation (Rohmer et al., 1993). Interestingly, formaldehyde transketolase shows high sequence similarity to its homolog TKT (ec. 2.2.1.1) in human (Sprenger and Pohl, 1999). Thus, our data support the possibility that TKT in human acts similarly to DHAS in yeast when under FA stress.

Fatty acid synthesis: In the current study screen, fatty acid synthesis (FAS) was demonstrated to be one of top-ranked metabolic pathways modulating FA cytotoxicity. Fatty acid synthase (FASN) is the key cytosolic enzyme in mammalian type I FAS required for *de novo* palmitate synthesis (Wu et al., 2016). Deletion of *FASN* was shown to enhance the tolerance to FA toxicity (Table S5B). On the other hand, the knockout of multiple genes involved in type II FAS, e.g. *OXSM*, *MCAT* and *MECR*, increased the sensitivity to FA stress (Table S5A). In mammalian cells, type II FAS system exists in mitochondria and is completely independent of the cytosolic FAS apparatus (Hiltunen et al., 2009; Liu et al., 2010). Our results revealed that mitochondrial type II FAS may play an important role in releasing FA-induced stress. Since the major sites of FAS are adipose tissue and the liver, more relevant cell lines and biochemistry studies are needed to further illuminate how the modulation of FAS systems influences FA cytotoxicity.

mTOR signaling pathway: The disruption of several genes related to mTOR signaling increase the cellular resistance to 150 μM FA treatment at day 20. These included three genes from late endosomal/lysosomal adaptor, MAPK and mTOR activator (LAMTOR) family (*LAMTOR1*, *LAMTOR2* and *LAMTOR3*) as well as two genes encoding Rag complex (*RRAGA* and *RRAGC*) (Figure 7) which are involved in amino acid-induced relocalization of the mTOR signaling complex I (mTORC1) to the lysosomes and its subsequent activation of the mTOR signaling cascade (Paquette et al., 2018; Sancak et al., 2010). mTOR is a key component coordinately regulating the balance between cell growth and autophagy in response to cellular physiological conditions such as nutrient starvation or reduced growth factors as well as environmental stress, and specifically, autophagy induction is negatively regulated by mTORC1 (Jung et al., 2010; Kim et al., 2008). Autophagy plays a vital role in promoting cell survival via nutrient recycling and the clearance of damaged organelles or oxidized and aggregated proteins (Green and Levine, 2014). Cells in various solid tumor and leukemia models can be induced to respond to chemotherapy, radiation therapy and immunotherapy via the inhibition of autophagy (Sehgal et al., 2015). In the present study, the disruption of LAMTOR family genes (*LAMTOR1*, *LAMTOR2* and *LAMTOR3*) or Rag complex components (*RRAGA* and *RRAGC*) would be expected to inhibit mTOR signaling pathway, which in turn would enhance autophagy. Moreover, a recent study reported that FA exposure inhibited the expression of mTOR in rat testis (Fang et al., 2016) and our latest study in mice showed that FA may induce hematopoietic toxicity by increasing DNA damage and suppressing the mTOR pathway (Ge et al., 2020). Correspondingly, our current study suggests that FA also may enhance the autophagy via the downregulation of mTOR expression. In short, the inhibition of mTOR or disruption of

genes comprising LAMTOR family or Rag complex may enhance autophagy, enabling enhanced cellular survival and tolerance to FA stress.

Myeloid Leukemia: Our early review of epidemiologic studies and an IARC monograph have shown that exposure to FA in occupational settings is associated with an increased risk of lymphohematopoietic cancers, particularly myeloid leukemia (Cogliano et al., 2005; Zhang et al., 2009). A large cohort study (>25,000 workers) conducted by the National Cancer Institute (NCI) strongly indicated an association of FA exposure with myeloid leukemia (Hauptmann, 2003). Our previous study found that FA-exposed workers had lower blood counts and bone marrow toxicity and FA exposure in human myeloid progenitor cells in vitro induced toxic effects (Zhang et al., 2010). In our current study, a set of genes involved in chronic myeloid leukemia was shown to significantly modulate FA cellular toxicity (Figure S5), providing additional biological plausibility to support the potential association of FA exposure with myeloid leukemia.

4.4 Variation in candidate genes identified on days 8 and 20

In our screen, multiple candidate genes identified at day 8 were not identified at day 20 (Table S7). We speculate that the longer-term exposure could ultimately overwhelm the compensatory mechanisms of the cells, even without mutations in key FA response genes, and thus the differential growth advantage between sensitive and more resistant mutant cells would not be as apparent. Alternatively, additional compensatory mechanisms could come into play during longer-term exposure which would similarly decrease the differences between the acutely sensitive mutants and the more resistant clones. For example, the knockout of multiple genes involved in DNA damage repair led cells to be more sensitive to FA exposure at day 8 but some of these genes, e.g. *ATM*, *FANCB* and *FANCE*, were not identified at day 20. In this case additional compensatory mechanisms may counteract the adverse effect induced by the deletion of DNA repair genes between days 8 and 20, making cells more resistant to FA and limiting the sensitivity. Further studies are needed to examine these hypotheses and are beyond the scope of the current study.

4.5 Study limitations

Even though we identified multiple genes and pathways known to modulate FA toxicity in the present genome-wide CRISPR screen, the screening approach has several potential weaknesses which may limit our ability to identify important functional modulators of FA toxicity. As a cancer cell line, K562 may not fully represent *in vivo* responses to FA exposure in healthy individuals. For example, previous work has demonstrated the importance of the p53 transcription factor in mediating apoptotic responses to FA (Wong et al., 2012). However, the K562 cell is a p53-null leukemic cell (Woo et al., 2016), and thus, most of apoptotic genes including p53 were not identified in our screen. K562 may contain multiple genetic mutations which may limit ability to identify particular cellular response pathways. However, due to the limited choices of primary cells compatible with CRISPR screening, we selected K562 cells to be consistent with our previous studies. In addition, transient inhibition of proteasome activity previously was shown to result in increased cytotoxicity in human cells exposed to biologically relevant doses of FA (Ortega-Atienza et al., 2015). At FDR <0.01, we did not identify the proteasome as a major modulator of

toxicity in this cell system which could reflect cell specific differences in toxicity or pre-existing defects in proteasome response in K562. However, a lower stringency (FDR<0.1) does identify candidates (data not shown).

In the current study, we identified multiple genes previously implicated in the cellular response to FA including the well characterized *ADH5*, *ESD* and *FANC* family genes. Further, previous studies in chicken DT-40 (Ridpath et al., 2007) and human PD20/PD20-D2 lymphoblastic cell lines (Ren et al., 2013) also found that increased sensitivity to FA exposure was conferred by multiple individual targeted disruptions of the *FANC* family genes, providing support for the validity of our current findings. We recognize that the mechanisms modulating FA toxicity revealed using different models and screening approaches may differ. Therefore, the role of the novel genes and potential pathways/mechanisms identified in our current study require further investigation.

5. Conclusions

In conclusion, using genome-wide CRISPR-based screening in human K562 cells, we confirmed genes and molecular mechanisms previously shown to modulate cellular toxicity to FA in various models and further revealed novel genes and pathways. Our findings have implications for understanding the potential mechanisms of underlying FA-associated carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

Barker S, Weinfeld M, Murray D, 2005 DNA-protein crosslinks: their induction, repair, and biological consequences. Mutat Res 589, 111–135. [PubMed: 15795165]

Bennett RK, Gonzalez JE, Whitaker WB, Antoniewicz MR, Papoutsakis ET, 2018 Expression of heterologous non-oxidative pentose phosphate pathway from Bacillus methanolicus and phosphoglucose isomerase deletion improves methanol assimilation and metabolite production by a synthetic Escherichia coli methylotroph. Metab. Eng 45, 75–85. [PubMed: 29203223]

Cogliano VJ, Grosse Y, Baan RA, Straif K, Secretan MB, Ghissassi FE, the Working Group for Volume 88, 2005 Meeting report: summary of IARC Monographs on formaldehyde, 2-butoxyethanol, and 1-tert - butoxy-2-propanol. Environ. Health. Perspect 113, 1205–1208. [PubMed: 16140628]

Cummins I, McAuley K, Fordham-Skelton A, Schwoerer R, Steel PG, Davis BG, Edwards R, 2006 Unique regulation of the active site of the serine esterase S-formylglutathione hydrolase. J. Mol. Biol 359, 422–432. [PubMed: 16626737]

de Graaf B, Clore A, McCullough AK, 2009 Cellular pathways for DNA repair and damage tolerance of formaldehyde-induced DNA-protein crosslinks. DNA Repair (Amst) 8: 1207–1214. [PubMed: 19625222]

Deltour L, Foglio MH, Duester G, 1999 Metabolic deficiencies in alcohol dehydrogenase Adh1, Adh3, and Adh4 null mutant mice: overlapping roles of Adh1 and Adh4 in ethanol clearance and metabolism of retinol to retinoic acid. J. Biol. Chem 274, 16796–16801. [PubMed: 10358022]

Duong A, Steinmaus C, McHale CM, Vaughan CP, Zhang L, 2011 Reproductive and developmental toxicity of formaldehyde: A systematic review. Mutat. Res. Rev. Mutat. Res 728, 118–138.

Edenberg HJ, McClintick JN, 2018 Alcohol dehydrogenases, aldehyde dehydrogenases, and alcohol use disorders: a critical review. Alcohol. Clin. Exp. Res 42, 2281–2297. [PubMed: 30320893]

Fang J, Li D, Yu X, Lv M, Bai L, Du L, Zhou D, 2016 Formaldehyde exposure inhibits the expression of mammalian target of rapamycin in rat testis. Toxicol. Ind. Health 32, 1882–1890. [PubMed: 26229097]

Ge J, Yang H, Lu X, Wang S, Zhao Y, Huang J, Xi Z, Zhang L, Li R, 2020 Combined exposure to formaldehyde and PM2.5: Hematopoietic toxicity and molecular mechanism in mice. Environ. Int 144, 106050. [PubMed: 32861163]

Gonzalez CF, Proudfoot M, Brown G, Korniyenko Y, Mori H, Savchenko AV, Yakunin AF, 2006 Molecular basis of formaldehyde detoxification: characterization of two S-formylglutathione hydrolases from Escherichia Coli, FrmB and YeiG. J. Biol. Chem 281, 14514–14522. [PubMed: 16567800]

Green DR, Levine B, 2014 To be or not to be? How selective autophagy and cell death govern cell fate. Cell 157, 65–75. [PubMed: 24679527]

Harms N, Ras J, Reijnders WN, van Spanning RJ, Stouthamer AH, 1996 S-formylglutathione hydrolase of Paracoccus denitrificans is homologous to human esterase D: a universal pathway for formaldehyde detoxification? J. Bacteriol 178, 6296–6299. [PubMed: 8892832]

Hauptmann M, 2003 Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries. J, Natl. Cancer Inst 95, 1615–1623. [PubMed: 14600094]

Hiltunen JK, Schonauer MS, Autio KJ, Mittelmeier TM, Kastaniotis AJ, Dieckmann CL, 2009 Mitochondrial fatty acid synthesis type II: more than just fatty acids. J. Biol. Chem 284, 9011–9015. [PubMed: 19028688]

Hsu PD, Lander ES, Zhang F, 2014 Development and applications of CRISPR-Cas9 for genome engineering. Cell 157, 1262–1278. [PubMed: 24906146]

International Agency for Research on Cancer, Weltgesundheitsorganisation (Eds.), 2012 IARC monographs on the evaluation of carcinogenic risks to humans, volume 100 F, chemical agents and related occupations: this publication represents the views and expert opinions of an IARC Working Group on the evaluation of carcinogenic risks to humans, which met in Lyon, 20 – 27 October 2009. IARC, Lyon.

Jung CH, Ro S-H, Cao J, Otto NM, Kim D-H, 2010 mTOR regulation of autophagy. FEBS Letters 584, 1287–1295. [PubMed: 20083114]

Kampmann M, 2018 CRISPRi and CRISPRa screens in mammalian cells for precision biology and medicine. ACS Chem. Biol 13, 406–416. [PubMed: 29035510]

Kaźmierczak M, Luczak M, Lewandowski K, Handschuh L, Czyż A, Jarmuż M, Gniot M, Michalak M, Figlerowicz M, Komarnicki M, 2013 Esterase D and gamma 1 actin level might predict results of induction therapy in patients with acute myeloid leukemia without and with maturation. Med. Oncol 30, 725. [PubMed: 24085543]

Kim E, Goraksha-Hicks Pankuri, Li L, Neufeld TP, Guan K-L, 2008 Regulation of TORC1 by Rag GTPases in nutrient response. Nat. Cell Biol 10, 935–945. [PubMed: 18604198]

Ladeira C, Viegas S, Carolino E, Gomes MC, Brito M, 2013 The influence of genetic polymorphisms in XRCC3 and ADH5 genes on the frequency of genotoxicity biomarkers in workers exposed to formaldehyde: influence of genetic polymorphisms in genotoxicity biomarkers. Environ. Mol. Mutagen 54, 213–221. [PubMed: 23355119]

Lan Q, Smith MT, Tang X, Guo W, Vermeulen R, Ji Z, Hu W, Hubbard AE, Shen M, McHale CM, Qiu C, Liu S, Reiss B, Beane-Freeman L, Blair A, Ge Y, Xiong J, Li L, Rappaport SM, Huang H, Rothman N, Zhang L, 2015 Chromosome-wide aneuploidy study of cultured circulating myeloid progenitor cells from workers occupationally exposed to formaldehyde. Carcinogenesis 36, 160–167. [PubMed: 25391402]

Langmead B, Trapnell C, Pop M, Salzberg SL, 2009 Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10, R25. [PubMed: 19261174]

Li H, Durbin R, 2009 Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754–1760. [PubMed: 19451168]

Liu H, Liu J-Y, Wu X, Zhang J-T, 2010 Biochemistry, molecular biology, and pharmacology of fatty acid synthase, an emerging therapeutic target and diagnosis/prognosis marker 21. Int. J. Biochem. Mol. Biol 1: 69–89. [PubMed: 20706604]

Liu Y, 2006 Studies on formation and repair of formaldehyde-damaged DNA by detection of DNA-protein crosslinks and DNA breaks. Front. Biosci 11, 991. [PubMed: 16146790]

McHale CM, Smith MT, Zhang L, 2014 Application of toxicogenomic profiling to evaluate effects of benzene and formaldehyde: from yeast to human: yeast and human toxicogenomic approaches. Ann. N.Y. Acad. Sci 1310, 74–83. [PubMed: 24571325]

Mitsui R, Sakai Y, Yasueda H, Kato N, 2000 A novel operon encoding formaldehyde fixation: the ribulose monophosphate pathway in the gram-positive facultative methylotrophic bacterium mycobacterium gastri MB19. J. Bacteriol 82, 944–948.

Nikitin A, Egorov S, Daraselia N, Mazo I, 2003 Pathway studio--the analysis and navigation of molecular networks. Bioinformatics 19, 2155–2157. [PubMed: 14594725]

North M, Gaytán BD, Romero C, De La Rosa VY, Loguinov A, Smith MT, Zhang L, Vulpe CD, 2016 Functional toxicogenomic profiling expands insight into modulators of formaldehyde toxicity in yeast. Front. Genet 7, 1–11. [PubMed: 26858746]

North M, Vulpe CD, 2010 Functional toxicogenomics: mechanism-centered toxicology. IJMS 11, 4796–4813. [PubMed: 21614174]

Panganiban RA, Park H-R, Sun M, Shumyatcher M, Himes BE, Lu Q, 2019 Genome-wide CRISPR screen identifies suppressors of endoplasmic reticulum stress-induced apoptosis. Proc. Natl. Acad. Sci. USA 116, 13384–13393. [PubMed: 31213543]

Paquette M, El-Houjeiri L, Pause A, 2018 mTOR pathways in cancer and autophagy. Cancers 10, 18.

Pontel LB, Rosado IV, Burgos-Barragan G, Garaycoechea JI, Yu R, Arends MJ, Chandrasekaran G, Broecker V, Wei W, Liu L, Swenberg JA, Crossan GP, Patel KJ, 2015 Endogenous formaldehyde is a hematopoietic stem cell genotoxin and metabolic carcinogen. Mol. Cell 60, 177–188. [PubMed: 26412304]

Potter AJ, Kidd SP, Edwards JL, Falsetta ML, Apicella MA, Jennings MP, McEwan AG, 2009 Esterase D is essential for protection of Neisseria gonorrhoeae against nitrosative stress and for bacterial growth during interaction with cervical epithelial cells. J. Infect. Dis 200, 273–278. [PubMed: 19527171]

Quievryn G, Zhitkovich A, 2000, Loss of DNA–protein crosslinks from formaldehyde-exposed cells occurs through spontaneous hydrolysis and an active repair process linked to proteosome function, Carcinogenesis 21, 1573–1580. [PubMed: 10910961]

Ortega-Atienza S, Green SE, Zhitkovich A, 2015 Proteasome activity is important for replication recovery, CHK1 phosphorylation and prevention of G2 arrest after low-dose formaldehyde. Toxicol. Appl. Pharm 286: 135–41.

Ortega-Atienza S, Wong VC, DeLoughery Z, Luczak MW, Zhitkovich A, 2016 ATM and KAT5 safeguard replicating chromatin against formaldehyde damage, Nucleic Acids Res 44, 198–209. [PubMed: 26420831]

Reczek CR, Birsoy K, Kong H, Martínez-Reyes I, Wang T, Gao P, Sabatini DM, Chandel NS, 2017 A CRISPR screen identifies a pathway required for paraquat-induced cell death. Nat. Chem. Biol 13, 1274–1279. [PubMed: 29058724]

Reingruber H, Pontel LB, 2018 Formaldehyde metabolism and its impact on human health. Curr. Opin. Toxicol 9, 28–34.

Ren X, Ji Z, McHale CM, Yuh J, Bersonda J, Tang M, Smith MT, Zhang L, 2013 The impact of FANCD2 deficiency on formaldehyde-induced toxicity in human lymphoblastoid cell lines. Arch. Toxicol 87, 189–196. [PubMed: 22872141]

Ridpath JR, Nakamura A, Tano K, Luke AM, Sonoda E, Arakawa H, Buerstedde J-M, Gillespie DAF, Sale JE, Yamazoe M, Bishop DK, Takata M, Takeda S, Watanabe M, Swenberg JA, Nakamura J,

2007 Cells deficient in the FANC/BRCA pathway are hypersensitive to plasma levels of formaldehyde. Cancer Res 67, 11117–11122. [PubMed: 18056434]

Rohmer M, Knani M, Simonin P, Sutter B, Sahm H, 1993 Isoprenoid biosynthesis in bacteria: a novel pathway for the early steps leading to isopentenyl diphosphate. Biochem. J 295, 517–524. [PubMed: 8240251]

Rosado IV, Langevin F, Crossan GP, Takata M, Patel KJ, 2011 Formaldehyde catabolism is essential in cells deficient for the Fanconi anemia DNA-repair pathway. Nat. Struct. Mol. Biol 18, 1432–1434. [PubMed: 22081012]

Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM, 2010 Ragulator-rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell 141, 290–303. [PubMed: 20381137]

Sanson KR, Hanna RE, Hegde M, Donovan KF, Strand C, Sullender ME, Vaimberg EW, Goodale A, Root DE, Piccioni F, Doench JG, 2018 Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nat. Commun 9, 5416. [PubMed: 30575746]

Schug ZT, 2018 Formaldehyde detoxification creates a new wheel for the folate-driven one-carbon "bi"-cycle. Biochem 57, 889–890. [PubMed: 29368500]

Sehgal AR, Konig H, Johnson DE, Tang D, Amaravadi RK, Boyiadzis M, Lotze MT, 2015 You eat what you are: autophagy inhibition as a therapeutic strategy in leukemia. Leukemia 29, 517–525. [PubMed: 25541151]

Shaham J, 2003 DNA-protein crosslinks and p53 protein expression in relation to occupational exposure to formaldehyde. Occup. Environ. Med 60, 403–409. [PubMed: 12771391]

Shaham J, Bomstein Y, Meltzer A, Kaufman Z, Palma E, Ribak J, 1996 DNA-protein crosslinks, a biomarker of exposure to formaldehyde— in vitro and in vivo studies. Carcinogenesis 17, 121–126. [PubMed: 8565120]

Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG, Zhang F, 2014 Genome-Scale CRISPR-Cas9 knockout screening in human cells. Science 343, 84–87. [PubMed: 24336571]

Shen H, McHale CM, Haider SI, Jung C, Zhang S, Smith MT, Zhang L, 2016 Identification of genes that modulate susceptibility to formaldehyde and imatinib by functional genomic screening in human haploid KBM7 cells. Toxicol. Sci 151, 10–22. [PubMed: 27008852]

Sobh A, Loguinov A, Stornetta A, Balbo S, Tagmount A, Zhang L, Vulpe CD, 2019a Genome-wide CRISPR screening identifies the tumor suppressor candidate OVCA2 as a determinant of tolerance to acetaldehyde. Toxicol. Sci 169, 235–245. [PubMed: 31059574]

Sobh A, Loguinov A, Yazici GN, Zeidan RS, Tagmount A, Hejazi NS, Hubbard AE, Zhang L, Vulpe CD, 2019b Functional profiling identifies determinants of arsenic trioxide cellular toxicity. Toxicol. Sci 169, 108–121. [PubMed: 30815697]

Sobh A, Vulpe C, 2019 CRISPR genomic screening informs gene–environment interactions. Curr. Opin. Toxicol 18, 46–53.

Sprenger GA, Pohl M, 1999 Synthetic potential of thiamin diphosphate-dependent enzymes. J. Mol. Catal. B: Enzym 6, 145–159.

Staab CA, Ålander J, Morgenstern R, Grafström RC, Höög J-O, 2009 The Janus face of alcoh ol dehydrogenase 3. Chem. Biol 178, 29–35.

Sundberg CD, Hankinson O, 2019 A CRISPR/Cas9 whole-genome screen identifies genes required for aryl hydrocarbon receptor-dependent induction of functional CYP1A1. Toxicol. Sci 170, 310–319. [PubMed: 31086989]

Tang X, Bai Y, Duong A, Smith MT, Li L, Zhang L, 2009 Formaldehyde in China: production, consumption, exposure levels, and health effects. Environ. Int 35, 1210–1224. [PubMed: 19589601]

Wei C, Wen H, Yuan L, McHale CM, Li H, Wang K, Yuan J, Yang X, Zhang L, 2017 Formaldehyde induces toxicity in mouse bone marrow and hematopoietic stem/progenitor cells and enhances benzene-induced adverse effects. Arch. Toxicol 91, 921–933. [PubMed: 27339418]

Whitaker WB, Sandoval NR, Bennett RK, Fast AG, Papoutsakis ET, 2015 Synthetic methylotrophy: engineering the production of biofuels and chemicals based on the biology of aerobic methanol utilization. Curr. Opin. Biotech 33, 165–175. 10.1016/j.copbio.2015.01.007 [PubMed: 25796071]

Wong VC, Cash HL, Morse JL, Lu S, Zhitkovich A, 2012 S-phase sensing of DNA-protein crosslinks triggers TopBP1-independent ATR activation and p53-mediated cell death by formaldehyde. Cell Cycle (Georgetown, Tex.) 11: 2526–2537.

Woo SM, Choi YK, Kim AJ, Cho SG, Ko SG, 2016 p53 causes butein-mediated apoptosis of chronic myeloid leukemia cells. Mol. Med. Rep 13, 1091–1096. [PubMed: 26676515]

Wu X, Dong Z, Wang CJ, Barlow LJ, Fako V, Serrano MA, Zou Y, Liu J-Y, Zhang J-T, 2016 FASN regulates cellular response to genotoxic treatments by increasing PARP-1 expression and DNA repair activity via NF-κB and SP1. Proc. Natl. Acad. Sci. USA 113, E6965–E6973. [PubMed: 27791122]

Xia P, Zhang X, Xie Y, Guan M, Villeneuve DL, Yu H, 2016 Functional toxicogenomic assessment of triclosan in human hepG2 cells using genome-wide CRISPR-Cas9 screening. Environ. Sci. Technol 50, 10682–10692. [PubMed: 27459410]

Yamazoe M, Sonoda E, Hochegger H, Takeda S, 2004 Reverse genetic studies of the DNA damage response in the chicken B lymphocyte line DT40. DNA Repair 3, 1175–1185. [PubMed: 15279806]

Ye X, Ji Z, Wei C, McHale CM, Ding S, Thomas R, Yang X, Zhang L, 2013 Inhaled formaldehyde induces DNA-protein crosslinks and oxidative stress in bone marrow and other distant organs of exposed mice: formaldehyde inhalation induces distant toxicity in mice. Environ. Mol. Mutagen 54, 705–718. [PubMed: 24136419]

Zhang L, Freeman LEB, Nakamura J, Hecht SS, Vandenberg JJ, Smith MT, Sonawane BR, 2010 Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. Environ. Mol. Mutagen 51, 181–191. [PubMed: 19790261]

Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT, 2009 Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. Mutat. Res. Rev. Mutat. Res 681, 150–168.

Zhang L, Tang X, Rothman N, Vermeulen R, Ji Z, Shen M, Qiu C, Guo W, Liu S, Reiss B, Freeman LB, Ge Y, Hubbard AE, Hua M, Blair A, Galvan N, Ruan X, Alter BP, Xin KX, Li S, Moore LE, Kim S, Xie Y, Hayes RB, Azuma M, Hauptmann M, Xiong J, Stewart P, Li L, Rappaport SM, Huang H, Fraumeni JF, Smith MT, Lan Q, 2010 Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells. Cancer Epidemiol. Biomarkers Prev 19, 80–88. [PubMed: 20056626]

Highlights

- Multiple genes related to FA cellular toxicity were identified using genome-wide CRISPR
- FA metabolism and the Fanconi anemia pathway were confirmed to modulate FA tolerance
- New genes in one-carbon metabolism were found to be associated with FA toxicity
- Novel pathways including fatty acid synthesis and mTOR signaling were discovered to modulate FA toxicity

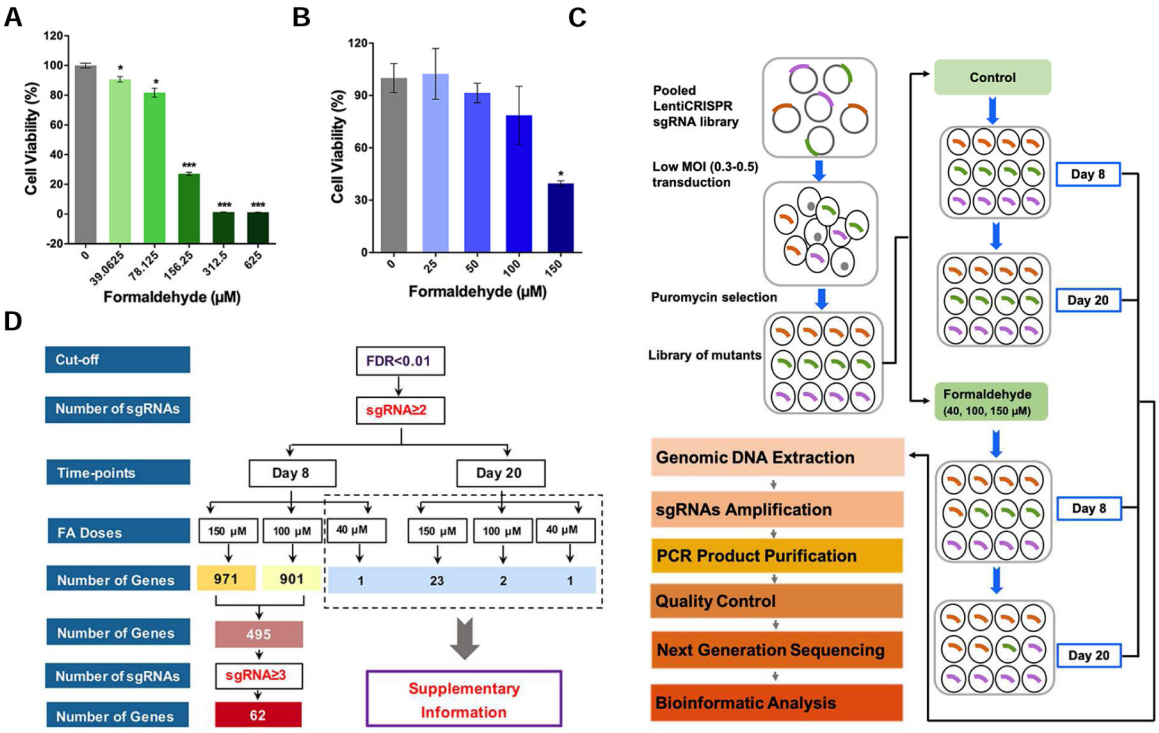


Figure 1.

Overview of the CRISPR-based functional screening approach. A. Cell viability test of a gradient of FA concentrations in K562 cells after 72 h in culture (1st experiment). Data are represented as mean±SEM (n=3). B. Cell viability test of multiple concentrations of FA in K562 cells after 72 h in culture (2nd experiment). Data are represented as mean±SEM (n=3). C. The workflow of the CRISPR screening process. D. Criteria to filter genes identified by edgeR.

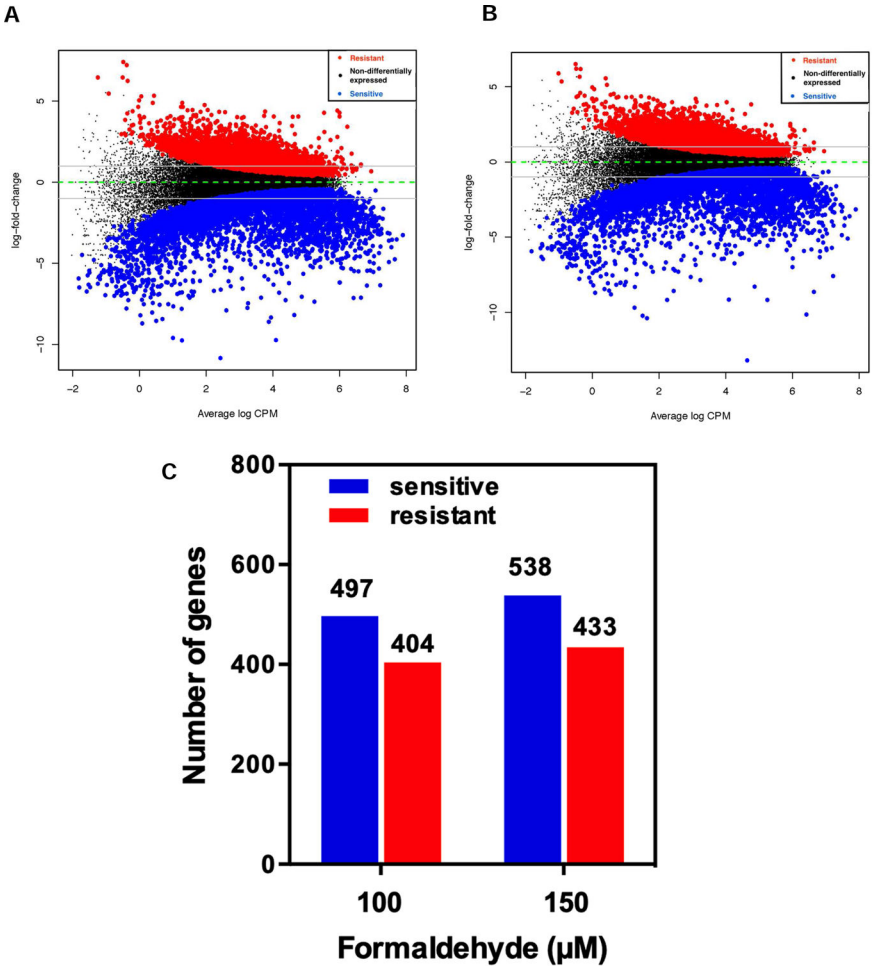


Figure 2.

Differential sgRNAs and candidate genes modulating FA cytotoxicity. A. Scatter plot showing the fold change of the enriched (log2FC>0) and the depleted (Log2FC<0) sgRNAs in the 100 μM FA exposure group compared to vehicle control (FDR<0.05). B. Scatter plot showing the fold change of the enriched (log2FC>0) and the depleted (Log2FC<0) sgRNAs in 150 μM FA exposure group compared to vehicle control (FDR<0.05). C. Number of sensitive/resistant genes with at least 2 sgRNAs in 100 μM and 150 μM FA at day 8 analyzed by edgeR (FDR<0.01).

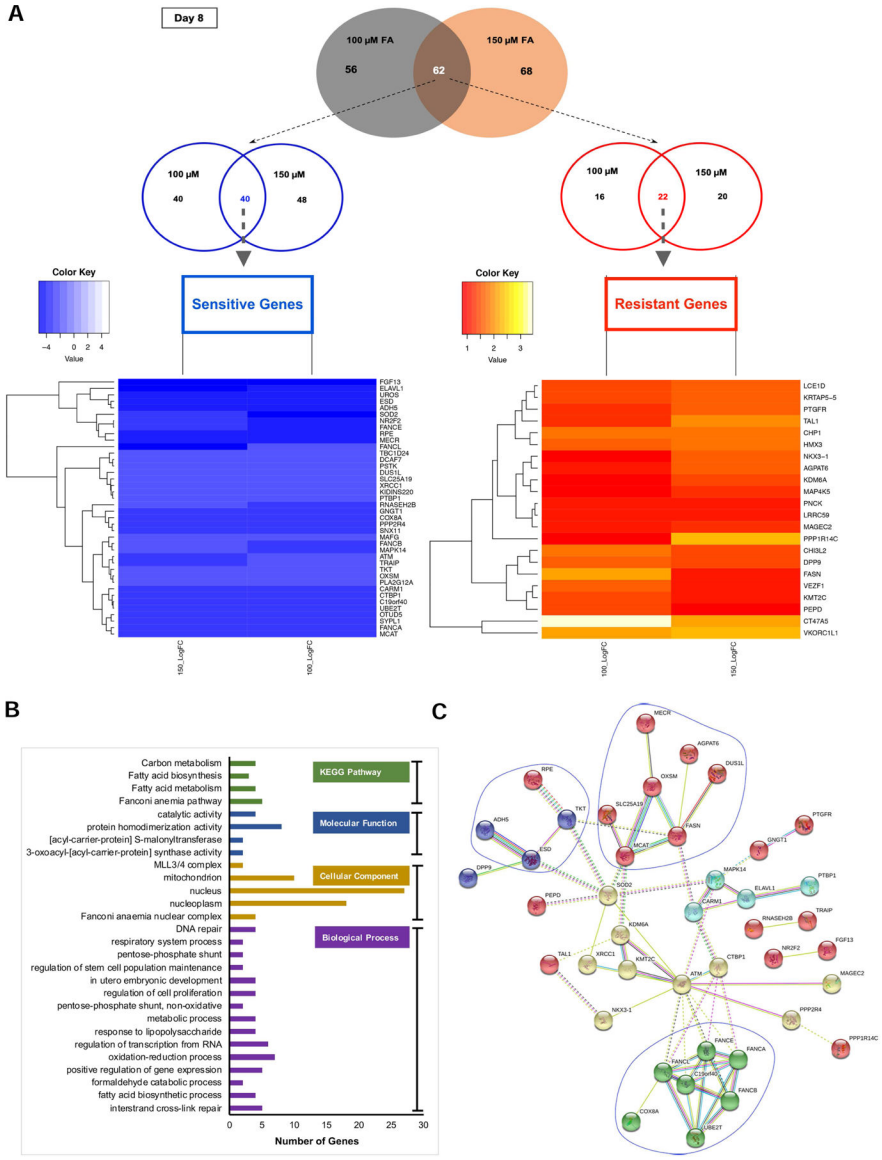


Figure 3.

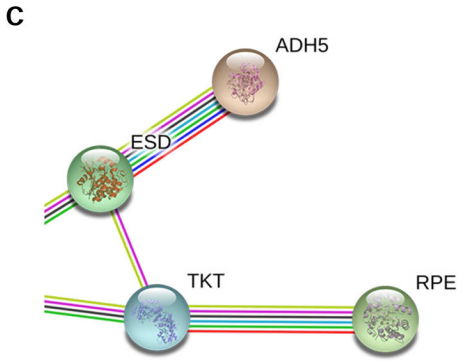
Functional characteristics of the 62 candidate genes identified in both 100 μM and 150 μM FA exposure at day 8 (FDR<0.01). A. Venn plot and heatmaps showing both sensitive (blue) and resistant (red) genes with at least 3 sgRNAs in same direction. B. Functional enrichment analysis showing the top pathways and biological processes modulating FA toxicity. C. Clustering analysis of the 62 candidate genes.

A

Gene	Gene Name
ADH5	alcohol dehydrogenase 5 (class III), chi polypeptide
ESD	esterase D
TKT	transketolase
RPE	ribulose-5-phosphate-3-epimerase

B

Functional Enrichment		
Gene Ontology	GO: 0006081	cellular aldehyde metabolic process
	GO: 0110095	cellular detoxification aldehyde
	GO: 0046294	formaldehyde catabolic process
	GO: 0046292	formaldehyde metabolic process
Kegg Pathways	hsa01200	carbon metabolism
	hsa00030	pentose phosphate pathway



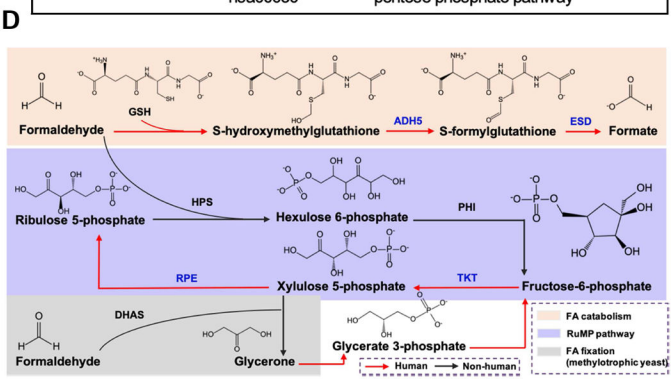


Figure 4.

Gene and pathway analysis of FA metabolism. Data obtained from candidate genes overlapping between 100 μM and 150 μM FA treatment at day 8 (FDR<0.01). A. List of genes related to FA metabolism. B. Annotations of all listed genes. C. Clustering analysis showing the strong interaction between four top candidate genes involved in FA metabolism. D. FA metabolism pathway showing the involvement of corresponding genes.

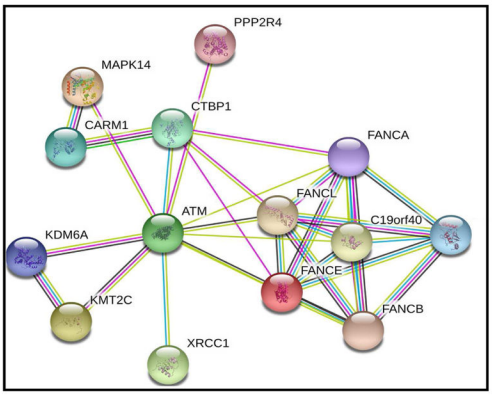
A

Gene	Gene Name
ATM	ATM serine/threonine kinase
CARM1	coactivator associated arginine methyltransferase 1
CTBP1	C-terminal binding protein 1
C19orf40	Fanconi anemia core complex-associated protein 24
FANCA	Fanconi anemia complementation group A
FANCB	Fanconi anemia complementation group B
FANCE	Fanconi anemia complementation group E
FANCL	Fanconi anemia complementation group L
KDM6A	lysine demethylase 6A
KMT2C	lysine methyltransferase 2C
MAPK14	mitogen-activated protein kinase 14
PPP2R4	protein phosphatase 2 phosphatase activator
UBE2T	ubiquitin conjugating enzyme E2 T
XRCC1	X-ray repair cross complementing 1

B

Functional Enrichment		
Gene Ontology	GO: 0036297	interstrand cross-link repair
	GO: 0006974	cellular response to DNA damage stimulus
	GO: 0006281	DNA repair
	GO: 0090304	nucleic acid metabolic process
	GO: 0000077	DNA damage checkpoint
	GO: 0043240	fanconi anemia nuclear complex

C



D

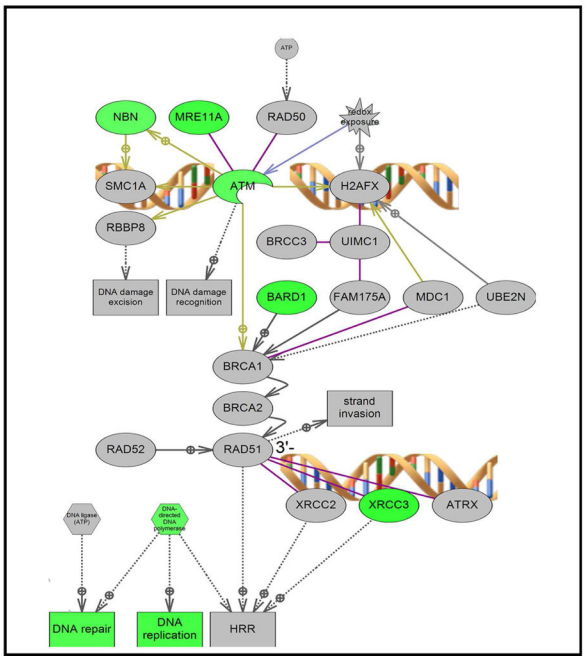


Figure 5.

Gene and pathway analysis of DNA damage and repair. Data obtained from candidate genes overlapping between 100 μM and 150 μM FA treatment at day 8 (FDR<0.01). A. List of genes involved in DNA damage and repair pathways. B. Annotations of all listed genes. C. Clustering analysis showing the connections between DNA repair associated genes, particularly the highly enriched Fanconi anemia complex components. D. DNA homologous repair pathway analyzed from 150 μM FA at day 8 by Pathway Studio.

A

Gene	Gene Name
MCAT	malonyl-CoA-acyl carrier protein transacylase
OXSM	3-oxoacyl-ACP synthase, mitochondrial
MECR	mitochondrial trans-2-enoyl-CoA reductase
FASN	fatty acid synthase
DUS1L	dihydrouridine synthase 1 like
SLC25A19	solute carrier family 25 member 19

B

Functional Enrichment		
Gene Ontology	GO: 0006633	fatty acid biosynthetic process
	GO: 0006631	fatty acid metabolic process
	GO: 0008610	lipid biosynthetic process
	GO: 0044281	small compound metabolic process
Kegg Pathways	hsa01212	fatty acid metabolism
	hsa00030	fatty acid biosynthesis

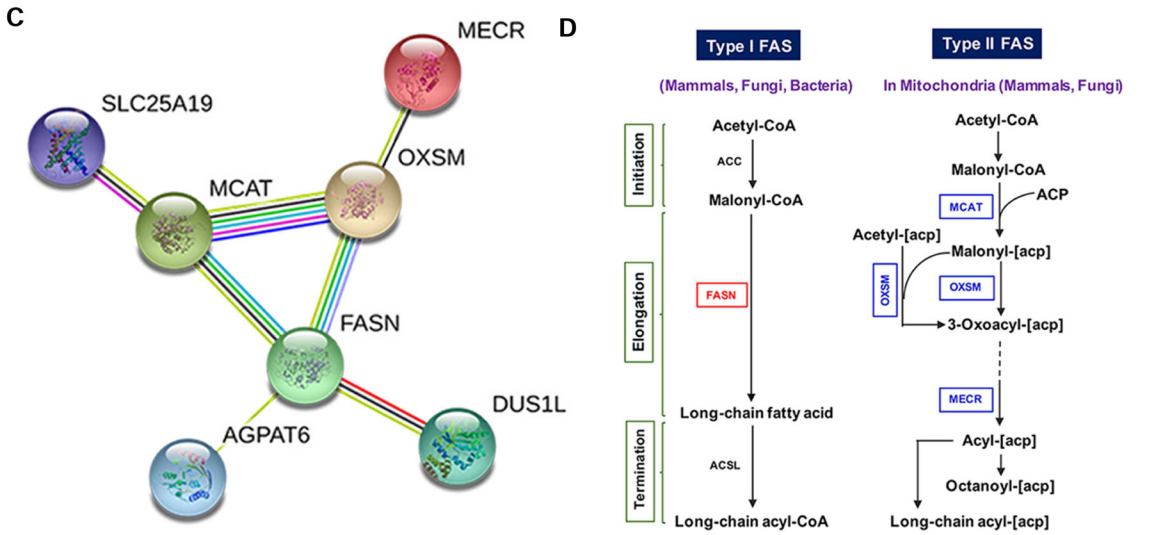


Figure 6.

Gene and pathway analysis of fatty acid synthesis. Data obtained from candidate genes overlapping between 100 μM and 150 μM FA treatment at day 8 (FDR<0.01). A. List of genes involved in fatty acid synthesis process. B. Annotations of all listed genes. C. Clustering analysis showing the connections between fatty acid synthesis related genes. D. The regulation of corresponding genes in the type I and II fatty acid synthesis pathways.

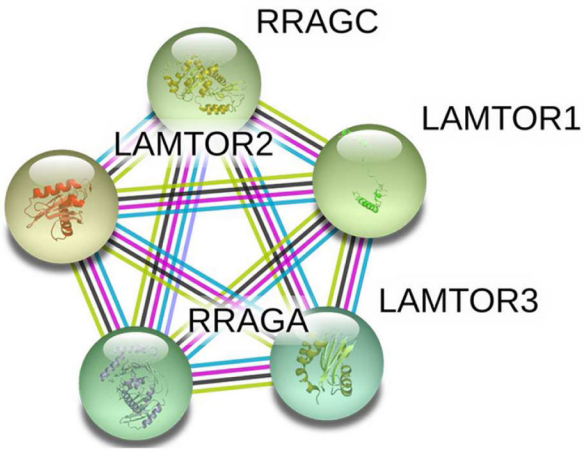
A

Gene	Gene Name
RRAGC	Ras related GTP binding C
RRAGA	Ras related GTP binding A
LAMTOR1	late endosomal/lysosomal adaptor, MAPK and mTOR activator 1
LAMTOR2	late endosomal/lysosomal adaptor, MAPK and mTOR activator 2
LAMTOR3	late endosomal/lysosomal adaptor, MAPK and mTOR activator 3

B

Functional Enrichment		
Gene Ontology	GO: 0032008	positive regulation of TOR signaling
	GO: 0016241	regulation of macroautophagy
	GO: 0071230	cellular response to amino acid stimulus
	GO: 0043410	postive regulation of MAPK cascade
Kegg Pathways	hsa04150	mTOR signaling pathway
	hsa04140	autophagy-animal

C



D

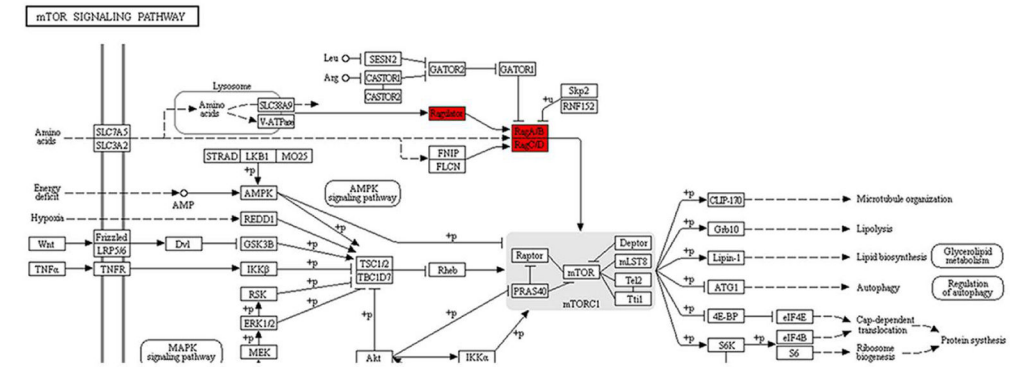


Figure 7.

Gene and pathway analysis of mTOR signaling pathway. Data obtained from candidate genes in 150 μM formaldehyde treatment group at day 20 (FDR<0.01). A. List of genes involved in TOR signaling pathway. B. Annotations of all listed genes. C. Clustering analysis showing the interactions between top resistant genes. D. Kegg pathway showing the involvement of corresponding genes in the mTOR signaling pathway.