Chemosphere 206 (2018) 643–654

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Disruption of ergosterol and tryptophan biosynthesis, as well as cell wall integrity pathway and the intracellular pH homeostasis, lead to mono-(2-ethylhexyl)-phthalate toxicity in budding yeast

Linghuo Jiang [a, *], Litong Wang [a], Tianshu Fang [a], Vassilios Papadopoulos [b, c]

[a] Laboratory for Yeast Molecular and Cell Biology, The Research Center of Fermentation Technology, School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, Shandong 255000, China
[b] The Research Institute of the McGill University Health Centre, Department of Medicine, McGill University, Montreal, Quebec H4A 3J1, Canada
[c] Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA

HIGHLIGHTS

- We identified 96 MEHP-sensitive yeast gene mutations from a genome-scale screen.
- Most mutants accumulated higher intracellular MEHP in response to MEHP treatment.
- Biosynthetic pathways of ergosterol and tryptophan are involved in MEHP tolerance.
- Cell wall integrity and pH homeostasis protect yeast cells from MEHP toxicity.
- We demonstrate that budding yeast is a useful for toxicogenomic analysis of MEHP.

ARTICLE INFO

Article history:
Received 19 November 2017
Received in revised form
13 April 2018
Accepted 12 May 2018
Available online 15 May 2018

Handling Editor: Jian-Ying Hu

Keywords:
MEHP
Ergosterol
Tryptophan
Cell wall integrity
Yeast
Genomics

ABSTRACT

Endocrine disrupting chemicals (EDCs) are substances in the environment, food, and consumer products that interfere with hormone homeostasis, metabolism or reproduction in humans and animals. One such EDC, the plasticizer di-(2-ethylhexyl)-phthalate (DEHP), exerts its function through its principal bioactive metabolite, mono-(2-ethylhexyl)-phthalate (MEHP). To fully understand the effects of MEHP on cellular processes and metabolism as well as to assess the impact of genetic alteration on the susceptibility to MEHP-induced toxicity, we screened MEHP-sensitive mutations on a genome-scale in the eukaryotic model organism *Saccharomyces cerevisiae*. We identified a total of 96 chemical-genetic interactions between MEHP and gene mutations in this study. In response to MEHP treatment, most of these gene mutants accumulated higher intracellular MEHP content, which correlated with their MEHP sensitivity. Twenty-seven of these genes are involved in the metabolism, twenty-two of them play roles in protein sorting, and ten of them regulate ion homeostasis. Functional categorization of these genes indicated that the biosynthetic pathways of both ergosterol and tryptophan, as well as cell wall integrity and the intracellular pH homeostasis, were involved in the protective response of yeast cells to the MEHP toxicity. Our study demonstrated that a collection of yeast gene deletion mutants is useful for a functional toxicogenomic analysis of EDCs, which could provide important clues to the effects of EDCs on higher eukaryotic organisms.

1. Introduction

Endocrine disrupting chemicals (EDCs) are substances in the environment, food and consumer products that interfere with hormone homeostasis, metabolism or reproduction through their interactions with hormone receptors or by modifying steroid biosynthetic pathways and metabolism (Diamanti-Kandarakis et al., 2009). EDCs can be classified into two broad categories, those such as pesticides originally designed to disrupt the endocrine systems of target species and those whose purpose was non-biological. The former is exemplified by nonsteroidal neuroendocrine agonists that have been developed as insecticides and

* Corresponding author.
E-mail address: linghuojiang@sdut.edu.cn (L. Jiang).

https://doi.org/10.1016/j.chemosphere.2018.05.069
0045-6535/

antiparasitic agents and are widely used in agriculture and aquaculture against harmful arthropods (Retnakaran et al., 2003). The latter includes a myriad of substances used in manufacturing and other processes.

One such group of manufacturing EDCs includes phthalates or phthalic acid esters (PAEs), which have been widely used since the 1920s as plasticizers in plastic products and have been detected throughout the environment worldwide (Zhang et al., 2017a; Net et al., 2015). Although the use of phthalate-free plastics is increasingly popular, these compounds are still present in a variety of industrial and consumer products, including food and beverage containers, electronics and medical devices (Mariana et al., 2016). One of the PAEs, di-(2-ethylhexyl)-phthalate (DEHP), is not covalently bound to the plastic and thus is gradually released into the environment. Therefore, both human and animal populations are in continuous contact with this compound through dietary intake, inhalation, and dermal and intravenous exposure via consumer and medical products. DEHP exerts its endocrine-disrupting property through its principal bioactive metabolite, mono-(2-ethylhexyl)-phthalate (MEHP) (Casals-Casas and Desvergne, 2011). For example, with medical interventions using PVC medical tubing, blood bags and medical devices, MEHP concentrations in the cord blood of new-born babies can reach 100 times those found in the general population, with levels up to 5 μM (Jones et al., 2016). Exposure to DEHP has harmful effects on human reproductive health and pregnancy outcome (Wen et al., 2015). Epidemiological studies and experimental research have even established the correlation between DEHP exposure and adverse cardiovascular conditions (Posnack, 2014). *In utero* DEHP exposure promotes local adipose tissue inflammation and chronic low-grade systemic inflammation in adult male offspring (Campioli et al., 2014). In the vertebrate model organism zebrafish, exposure to low concentrations of DEHP alters pericardial, head, and yolk morphology (Kinch et al., 2016).

DEHP affects sterol homeostasis in humans and animals. It causes an accumulation of lipid droplets in the mouse MA-10 Leydig tumor cell line (Dees et al., 2001). Low concentrations of MEHP induces gene expression profiles usually elicited by the human chorionic gonadotropin alone in the MA-10 Leydig cells, suggesting MEHP could interrupt the steroid biosynthesis in animals and humans (Giulivo et al., 2014; Fan et al., 2010). Combinatory exposure of MEHP with the phytoestrogen genistein, albeit not individually, to the MA-10 mouse tumor Leydig cells is able to increase levels of several neutral lipids and phospholipid classes, indicating a generalized deregulation of lipid homeostasis (Jones et al., 2016). DEHP exposure is also linked with the increased incidence of obesity and obesity-related disorders (Hao et al., 2013). In addition, it has been shown to suppress fetal and adult testosterone biosynthesis (Lee et al., 2017; Martinez-Arguelles et al., 2013; Culty et al., 2008).

In pubertal rats exposure to DEHP alters cholesterol balance and suppresses steroidogenesis (Botelho et al., 2009) and inhibits MA-10 Leydig cell steroidogenesis by targeting cholesterol transport (Zhou et al., 2013). Excess exposure to DEHP can exacerbate non-alcoholic fatty liver disease in Sprague Dawley rats and promotes lipid accumulation in hepatocytes via activating the SREBP-1c and PPARα-signaling pathway (Zhang et al., 2017b). Maternal exposure to DEHP has lasting effects on the physiological functions of the vascular system, adipose tissue, cholesterol metabolism and nerve system in mouse offspring (Lee et al., 2016). A recent study indicates that MEHP induces the formation of multinucleated germ cells in organ cultures of rat fetal and neonatal testes (Boisvert et al., 2016). Global gene expression analysis of prepubertal and adult adrenal glands indicate that *in utero* exposure to DEHP induces long-term changes in gene expression in the adult male adrenal gland, which might contribute to DEHP-induced abnormality in mice (Martinez-Arguelles et al., 2014). In addition, MEHP induces significant enrichment of cholesterol/lipid/steroid metabolism and apoptosis pathways, which is associated with mouse developmental toxicity (Robinson et al., 2012). Given the multiplicity of known effects of DEHP and its metabolite MEHP in animals, the ability to quickly identify the full range of possible gene-chemical interactions and affected cellular processes for this EDC, as well as others, would be extremely useful. Considering the complexity of mammalian systems, such an endeavor may be easier accomplished in a simple eukaryotic cell model organism such as *Saccharomyces cerevisiae.*

Collections of yeast gene deletion mutants have been successfully used in the characterization of disease gene functions, gene-chemical and pathway-chemical interactions (Zacchi et al., 2017; Marmiroli et al., 2016; Xiong et al., 2015; Zhao et al., 2013a, 2013b; Brenner, 2004). Up to now, there is no report on the use of *S. cerevisiae*, to study the effect of EDCs on cellular processes and metabolism.

We used *S. cerevisiae* to dissect gene-chemical interactions, which can help us understand the effects of EDCs on the metabolism and cellular processes in higher eukaryotes as well as assess the role of genetic alterations on the susceptibility to MEHP-induced toxicity. We have screened the yeast nonessential gene deletion library and identified a total of 96 genetic interactions between MEHP and gene mutations in this study. Interestingly, we have revealed that the biosynthetic pathways of ergosterol and tryptophan, as well as the cell wall integrity and the intracellular pH homeostasis are involved in the sensitivity of yeast cells to MEHP.

2. Materials and methods

2.1. Strains and media

A collection of homozygous diploid strains (the wild-type *S. cerevisiae* strain B4743 and its collected mutants derived from the S288C background) corresponding to deletions of each of non-essential 4757 genes was purchased from Invitrogen Inc. (Jiang et al., 2014). The haploid wild-type strain B4741 was tested for the MEHP sensitivity of yeast cells, but was not chosen for further experimentation (Supplemental Fig. 1). All yeast cells were grown at 30 °C in YPD medium (1% yeast extract, 2% peptone, 2% glucose). MEHP was purchased from Wako Pure Chemical Industries Ltd (Japan). A stock solution of 200 mM MEHP was prepared with pure ethanol.

2.2. Genome-wide screen for MEHP-sensitive gene deletion mutations

As a primary screen for MEHP-sensitive mutations in *S. cerevisiae*, we replicated the collection of homozygous diploid deletion mutants of non-essential 4757 genes using a steel replicator (Nalgene Nunc International) onto YPD plates supplemented with 1600 μM MEHP. This MEHP concentration was chosen after an initial test of MEHP toxicity to yeast cells, because supplementation of 3200 μM MEHP, but not up to 1600 μM MEHP, almost completely inhibited the growth of BY4743 and BY4741 (Supplemental Fig. 1). The solvent ethanol alone at a concentration of 1.6%, which is equivalent to that in YPD medium containing 3200 μM MEHP, would not affect the normal growth of the diploid wild type and its deletion mutants (Wu et al., 2016). It should be noted that this MEHP concentration is very high as compared to the MEHP toxicity in mammalian cells (Dees et al., 2001), but it was chosen in an effort to identify gene targets leading to toxicity in yeast cells. A mutant