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Low dose perfluorooctanoate exposure promotes cell proliferation in a human non-tumor liver cell line

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HIGHLIGHTS

- Differential expression of proteins induced by PFOA in HL-7702 was identified.
- Most of the differentially expressed proteins are related to cell proliferation.
- A low dose of PFOA stimulates HL-7702 cell proliferation.
- A high dose of PFOA inhibits HL-7702 cell proliferation.

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ABSTRACT

Perfluorooctanoate (PFOA) is a well-known persistent organic pollutant widely found in the environment, wildlife and humans. Medical surveillance and experimental studies have investigated the potential effects of PFOA on human livers, but the hepatotoxicity of PFOA on humans and its underlying mechanism remain to be clarified. We exposed a human liver cell line (HL-7702) to 50 μM PFOA for 48 h and 96 h, and identified 111 significantly differentially expressed proteins by iTRAQ analysis. A total of 46 proteins were related to cell proliferation and apoptosis. Through further analysis of the cell cycle, apoptosis and their related proteins, we found that low doses of PFOA (50–100 μM) promoted cell proliferation and numbers by promoting cells from the G1 to S phases, whereas high doses of PFOA (200–400 μM) led to reduced HL-7702 cell numbers compared with that of the control mainly due to cell cycle arrest in the G0/G1 phase. To our knowledge, this is the first report on the promotion of cell cycle progression in human cells following PFOA exposure.

1. Introduction

Due to their chemical and thermal resistance and ability to repel both oil and water, perfluoroalkyl acids (PFAAs) have been widely used in a variety of industrial and consumer applications over the past 50 years, including surfactants, lubricants, adhesives, paints, fire-fighting foams and cosmetics [1]. Paradoxically, their thermal and chemical persistence also results in a high degree of environmental persistence and bioaccumulation. As a result, PFAAs have been found in the ocean, air, wildlife and humans worldwide [2,3]. Perfluorooctanoate (PFOA) is one of the most often reported and discussed PFAAs in the scientific literatures [3–6]. The population geometric mean for serum PFOA has been reported to be 32.91 ng/mL in districts with PFOA contaminated water [7] which was about 5–8 fold higher than previously reported for a representative American population based on NHANES (National Health and Nutrition Examination Survey) data from 1999 to 2000 and 2007 to 2010, respectively [8,9]. The highest levels of PFOA were described in ammonium perfluorooctanoate production workers, with serum PFOA concentrations of 114,100 ng/mL [10].

Studies in rats and monkeys suggest that PFOA is correlated with multiple toxicities, including hepatotoxicity, carcinogenicity, immunotoxicity and developmental effects [6,11–13]. The liver has been demonstrated to be a primary bioaccumulative and target organ in rodent toxicity studies [14]. Liver toxicities such as liver enlargement, liver tumor, hepatocellular hypertrophy and vacuolation and lipid metabolism disorder are reportedly initiated by the activation of peroxisome proliferator-activated receptors (PPARs) [11,15]. Additionally, some rodent studies using knockout mice suggest that PPARs may not be the only pathways activated by PFOA [16–18]. Recently, human cell line *in vitro* studies showed that PFOA

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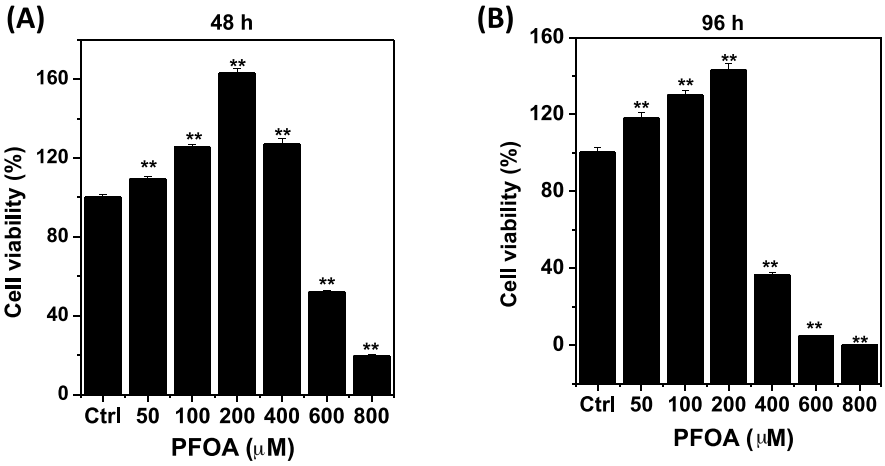

Fig. 1. Cell viability of HL-7702 cells exposed to PFOA for 48 h (A) and 96 h (B). Data are presented as means ± SE of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$.

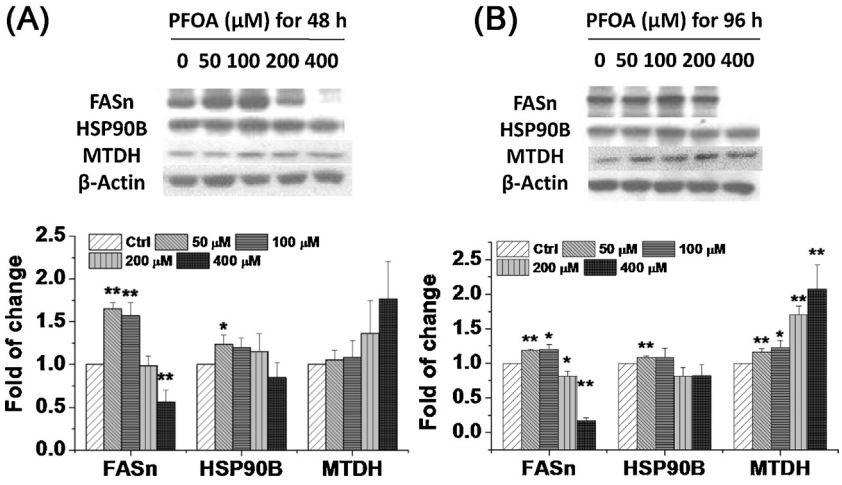

Fig. 2. Western blot verification of proteins identified in the iTRAQ results following PFOA exposure for 48 h (A) and 96 h (B). Left panel shows representative blots from three experiments. Right panel shows mean levels of protein bands compared with the control. Data are means ± SE ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$ compared with control.

can induce reactive oxygen species [19], inhibit HNF4α expression [20], perturb the cell cycle and induce apoptosis and DNA breaks in HepG2 cells [19,21]. An induction of glutathione- S-transferase Pi (GSTP) aberrant methylation by PFOA was also observed in human L02 cells [22]. Moreover, epidemiological and medical surveillance studies have reported inconsistent associations between PFOA and liver enzymes. Transaminase (ALT) levels, a marker of hepatocellular damage, were found to be positively associated with PFOA concentrations in some occupational and general population studies [23,24], but not in others [25].

Additionally, PFOA has a longer elimination half-life in humans (about 3.8 years) than that in rodents (4–19 days) [6], and thus might have higher accumulation. In humans, however, the causal biochemical mechanisms of hepatic toxicity after PFAA exposure are not clearly defined. PPARα-related responses in human liver cells are quantitatively and qualitatively different than those observed in rodents, therefore rodent data may not be a relevant indicator of PFAA risk in humans [26]. Thus, human hepatotoxicity to PFOA and its mechanism of action needs further research.

Proteomic technologies have been successfully used in toxicology studies. Isobaric tags for relative and absolute quantitation (iTRAQ) is one of the most widely used approaches. Using iTRAQ combined with 2D liquid chromatography and tandem mass spectrometry (2DLC–MS/MS), we identified the differentially expressed proteins in a human non-tumor liver cell line (HL-7702) after PFOA exposure. Through bioinformatic analysis, we found over one hundred proteins were related to cell proliferation and apoptosis. Therefore, we further detected the cell cycle by flow cytometer analysis and analyzed the protein levels of its regulators by western blot to confirm the effect and explore the mechanisms of PFOA on human liver cells.

2. Materials and methods

2.1. Cell culture, treatment and cell viability assay

HL-7702 cells are immortalized non-tumor cells derived from primary normal human hepatocytes, expressing a distinct ultrastructure compared to hepatic carcinoma cells and are considered as an ideal *in vitro* model of a Chinese nonmalignant liver [27]. The HL-7702 cells were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The cells were cultured in RPMI-1640 complete culture medium containing 2.05 mM L-glutamine, 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin and 100 g/mL streptomycin at 5% CO_2 and 37 °C.

For cell viability assay, cells were seeded on 96-well plates at a density of 1×10^4 cells/well and 3×10^3 cells/well for 48 h and 96 h exposure, respectively. After 24 h cultured, the cells were treated