Original Article

Pathway of 3-MCPD-induced apoptosis in human embryonic kidney cells

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ABSTRACT — 3-Chloropropane-1,2-diol (3-MCPD) is a heat-produced contaminant formed during the preparation of soy sauce worldwide. The present investigation was conducted to determine the molecular aspects of 3-MCPD toxicity on human embryonic kidney cells (HEK293). Cell viability and apoptosis were assessed in response to exposure to 3-MCPD using the MTT assay and high-content screening (HCS). DNA damage, intracellular reactive oxygen species (ROS) and apoptosis-related proteins were evaluated. Genes related with apoptosis were detected by qPCR-array for further understanding the 3-MCPD induced cell apoptosis signaling pathway. Our results clearly showed that 3-MCPD treatment inhibits cell proliferation and reactive oxygen species generation. qPCR-array indicated that nine apoptotic genes were up-regulated more than 2-fold and six down-regulated more than 2-fold. Genes associated with the mitochondrial apoptotic pathway, especially BCL2 family genes, changed significantly, indicating that the mitochondrial apoptotic pathway is activated. Death receptor pathway-related genes, TNFRSF11B and TNFRSF1A, changed significantly, indicating that the death receptor pathway is also activated, resulting in the inhibition of cell growth and proliferation as well as induction of apoptosis. To sum up, the experiment results indicated that 3-MCPD induced HEK293 cell toxicity through the death receptor pathway and mitochondrial pathway.

Key words: 3-Chloropropane-1,2-diol, HEK293 cell, Cell apoptosis, Genetic pathway, Cellular toxicity

INTRODUCTION

3-Chloro-1,2-propanediol (3-MCPD) is one of the chloropropanol chemicals formed by the reaction between hydrochloric acid and residual vegetable fat in the process of producing acid-hydrolyzed vegetable protein (HVP) (Crews *et al.*, 2003). In recent years, HVP has been increasingly used as a common savory ingredient widely added to soy sauce and various composite seasonings (Nyman *et al.*, 2003). Corresponding to the increase in HVP addition, 3-MCPD has been detected in a wide range of blended soy sauces (Weißhaar, 2011). 3-MCPD has also been detected in various heat-processed foods, including cereal-derived products like bread crust, toast and biscuits, malt-derived products, coffee, and grilled cheese, as well as smoke-treated products (Bakhiya *et al.*, 2011; Schallschmidt *et al.*, 2012; Teng and Wang, 2014). The toxicity of 3-MCPD is well-documented and includes

neurotoxicity (Cavanagh and Nolan, 1993), nephrotoxicity (Velisek *et al.*, 1980), genotoxicity (Lynch *et al.*, 1998), immunotoxicity (Lee *et al.*, 2004) and reproductive toxicity (Lynch *et al.*, 1998). Recently, 3-MCPD was classified by the IARC as group 2B, “possibly carcinogenic to humans” (Bakhiya *et al.*, 2011). The European Union (EU) established a limit level of 20 µg kg⁻¹ for 3-MCPD in HVP and soy sauce (Crews *et al.*, 2003). The cell death signaling pathway of 3-MCPD is not well-established and could be due to the inhibition of protein and nucleic acid synthesis and to a genotoxic action.

Oxidative damage can severely compromise cell homeostasis and viability. It also induces a variety of cellular responses through the generation of secondary reactive species, ultimately leading cells to program their own death (Richter *et al.*, 1988; Sies, 2013). The most common and well-defined form of programmed cell death is apoptosis, which is a physiological cell-suicide pro-

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gramme that is essential for embryonic development (Campos-Ortega and Hartenstein, 2013), immune-system function (Abbas *et al.*, 2012) and the maintenance of tissue homeostasis (Sonnenberg *et al.*, 2011) in multi-cellular organisms. Deregulation of apoptosis has been implicated in numerous pathological conditions, including neurodegenerative diseases (Radi *et al.*, 2014), autoimmunity and cancer (Rabinovich and Croci, 2012).

There is a widespread interest in the cellular mechanisms used by organisms to cope with the disruption of homeostasis (Ling *et al.*, 2013). At the cellular level, the presence of toxic chemicals induces numerous biochemical mechanisms including temporary modifications in gene expressions (Lim *et al.*, 2012). One of the key cellular responses to toxicant exposure, which could potentially be used as an early marker of toxicity, is caspase family protein (Kalia and Bansal, 2009).

Apoptosis, the programmed cell death in eukaryotic cells, plays a vital role in multi-celled tissue-forming organisms (Rasheva and Domingos, 2009). It is particularly important with respect to the elimination of damaged cells. Compared with necrosis, a passive process, apoptosis is an active metabolic performance which is executed along determined pathways (Günther *et al.*, 2013). Because of this, the cell is subject to strict (self) control and is eliminated without affecting neighboring cells (Chopra *et al.*, 2010). Three main components are involved in apoptosis signal transduction: mitochondrial pathway (Zhang *et al.*, 2010), death receptor pathway (Nebbioso *et al.*, 2011) and endoplasmic reticulum pathway (Zhang and Kaufman, 2008). All pro-apoptotic molecules exist in intact cells and do not have to be synthesized newly (Ashkenazi, 2008). Most of these molecules are found either as inactive precursors or play other metabolic roles in different organelles (Moll *et al.*, 2005).

In the present study, 3-MCPD was evaluated for its cytotoxicity in rat kidney HEK-293 cells. We have monitored the effects of 3-MCPD on (i) cell viability, (ii) DNA damage, (iii) oxidative damage and (iv) cell death signaling pathway.

MATERIALS AND METHODS

Chemicals

3-Chloro-1,2-propandiol (3-MCPD, 97%) were purchased from J&K China Chemical Ltd. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS) and phosphate buffer saline (PBS) were obtained from Gibco (Grand Island, NY, USA). Trizol reagent was purchased from Sangon Biological Technology (Shanghai, China). The phosphate buffer solution (0.1 M K_2HPO_4 /

NaH_2PO_4 , pH 7.29) used in this study was obtained from Sigma. All other chemicals used were of HPLC grade. Deionized water used for all experiments was purified with a Milli-Q system (Millipore, MA, USA).

Cell culture

HEK293 cells (Chinese Academy of Sciences, Shanghai, China) were cultured in a flask in DMEM medium that was supplemented with 10% fetal calf serum, penicillin (100 μ g/mL), and streptomycin (100 μ g/mL) at 37°C in a humidified atmosphere containing 5% CO_2 . At the growth retardation stage after 3 d, the cells reached the logarithmic phase of growth, after which they were used for experiments.

HEK293 cell viability assays

Cell viability was assessed in response to exposure to 3-MCPD using the Image Xpress Micro XLS (Molecular Devices, CA, USA), which simultaneously measures cell viability and cytotoxicity, based on the Annexin V-FITC Apoptosis Detection Kit (Beyotime Co., Ltd., Shanghai, China). HEK293 cells were seeded at a density of 250,000 cells/well in a 96-well plate. Cells were fed with dosed media, for 24 hr. After the media removed, the cells were washed 3 times with PBS buffer. Annexin V-FITC, propidium iodide (PI) and 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) dyes were used followed the specification. Each experiment was replicated at least three times. Cell viability was calculated based on the fluorescence intensity of the 3 kinds of dyes. The green fluorescence intensity is representative of the Annexin V-FITC, indicating the early apoptosis; the red fluorescence intensity for PI, indicating the late apoptotic (marker the cells without unbroken cytomembrane); and the blue fluorescence intensity for DAPI, indicating the cell number (marker the cells weather the cytomembrane broken or not). FACS Calibur flow cytometer (Becton Dickinson, NJ, USA) was applied for quantitative analysis of the relationship between the concentration of 3-MCPD and apoptosis.

Oxidative stress status

Reactive oxygen species (ROS) was detected with the reactive oxygen species assay kit (Beyotime Biotechnology) according to the manufacturer's instructions (ID: S0033). We used 2',7'-dichloro-fluorescein diacetate (DCFH-DA) to detect the intracellular ROS. Cells were seeded at a density of 1×10^6 cells/well in a 6-well plate and incubated for 12 hr. After treatment with various concentrations of 3-MCPD (0, 5, 10, 20 mM) for 24 hr, we washed the cells with PBS twice and loaded them with

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10 μ M DCFH-DA in serum-free medium for 60 min at 37°C without light, followed by three more rinses in PBS. We monitored the formation of the fluorescent-oxidized DCF derivative using FACS Calibur flow cytometer, with excitation at 485 nm and emission at 525 nm. Using the median fluorescence intensity of 20,000 cells, we quantified ROS generation.

Comet assay

Using the OxiSelect™ 96-Well Comet Assay Kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions, we trypsinized cells treated with 3-MCPD for 24 hr, then diluted with ice-cold PBS. The positive controls here are 100 μ M H₂O₂-treated cells. We mixed 3-MCPD- and H₂O₂-treated cells with low-melting-point agarose to coat the slides. Next, we treated the slides with lysis buffer and alkaline solution, electrophoresed them under neutral conditions, and stained them with DNA dye. For analysis, we selected two slides per concentration and a total of 50 comets per group (25 cells per slide). CASP image analysis software was applied to the captured images. We measured the olive tail moment (OTM) (Kent *et al.*, 1995), calculated as comet tail moment length multiplied by the relative DNA content of the tail, as an indicator of DNA damage.

Caspase-3, caspase-8 and caspase-9 activity assay

Caspase-3, caspase-8 and caspase-9 activity of HEK 293 cells treated with different concentration of 3-MCPD (0 mM, 5 mM, 10 mM and 20 mM) for 24 hr were measured using a commercially available kit (Caspase 3, 8 and 9 Activity Assay Kit, Beyotime), according to the manufacturer's instructions. In the presence of active caspase-3, caspase-8 and caspase-9 cleavage and release of p-nitroanilide (pNA) from the substrate occurs. Free pNA produces a yellow color detected by a spectrophotometer at 405 nm. A standard curve was realized in order to determine the correspondence between absorbance and pNA concentration. Results were expressed as caspase-3, caspase-8 and caspase-9 specific activity (pmol pNA/hr/ μ g protein) calculated according to the manufacturer. The results were expressed as fold increase in caspase activity of apoptotic cells over that of non-induced cells. The sequence of the primers for the apoptotic gene, Bcl-2, Bax, P53 and CYC, *et al.*, is presented in Table S1.

Protein extraction and Western blot analysis

Western blots were carried out by the method of Towbin (Blake *et al.*, 1984). Cells (2×10^6) were treated, trypsinized and the protein was extracted using mamma-

lian cell lysis buffer. The protein was estimated by the Lowry method. All samples were loaded on an equal protein basis and analyzed by 10% SDS-PAGE gel. The separated proteins were transferred onto a nitrocellulose membrane and probed with monoclonal primary antibodies at 1:200 dilution. HRP conjugated rabbit anti-mouse antibody was used at a 1:5000 dilution. To check for equal protein loading, GAPDH was used as an internal control and developed with GAPDH primary antibody. Densitometric analysis was carried out using UN-SCAN-IT image digitalizing software.

Detection of genes related with apoptotic by qPCR-array

Sample RNA extracted from HEK 293 cells were conducted for the process of cDNA synthesis, after which, real-time PCR was used for detection. Briefly, 1050 μ L 2X SuperArray PCR master mix solution, 105 μ L cDNA and 945 μ L DD water were added in an EP tube. Tear off the member of PCR array carefully, 20 μ L of the mixed solution was added to each cell.

Statistical analysis

Quantitative data is expressed as mean $\pm$ S.D. and analyzed by one-way ANOVA. Comparison between the groups was made by analyzing data with post-hoc method. Statistical significance was set at a level of $P < 0.05$. Some statistical analyses were conducted using the Statistical Program for Social Sciences 13.0 software program (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

3-MCPD induces cell death

The cellular morphology treated with 3-MCPD at the indicated concentrations for 24 hr was evaluated by microscope, as shown in Fig. 1A. With the increasing concentration of 3-MCPD, the change of cellular morphology observed was of difference obviously. The cellular morphology, treated with 40 mM 3-MCPD, reflected visible inhibition of cell proliferation. The inhibition of cell proliferation treated with different 3-MCPD for 12 hr, 24 hr and 36 hr was evaluated by MTT assay, in Fig. 1B. As observed in the figure, the 40 mM 3-MCPD induced approximately 70% inhibition of cell proliferation; and the time effect of 3-MCPD was distinctly noticed. The IC₅₀ value as determined after 24 hr of cell treatment from the viability curve was approximately 20 mM. Fig. 1C shows the cell viability assessed in response to exposure to 3-MCPD using the Image Xpress Micro XLS, which simultaneously measures cell viability and cyto-

Fig. 1. Cytotoxic effect of 3-MCPD on HEK293 cells. The cellular morphology treated with 3-MCPD at the indicated concentrations for 24 hr was evaluated by microscope (A); and the inhibition of cell proliferation treated with 3-MCPD at the indicated concentrations for 12 hr, 24 hr and 36 hr was detected by MTT assay and expressed as percentages of control. Control value was taken as 0%. Data are expressed as the mean $\pm$ S.D. (B). The apoptosis experiment was conducted the HCS for evaluating the toxicity of 3-MCPD at the indicated concentrations for 24 hr (C) and the apoptosis rate was calculated. Control value was taken as 0%. Data are expressed as the mean $\pm$ S.D., Dunnett post test was applied for comparison all dosed-groups with control group, * for $P < 0.05$, and ** for $P < 0.01$.

toxicity, based on the Annexin V-FITC Apoptosis Detection Kit (Zhang *et al.*, 1997). Cells fed with 10 mM for 24 hr exhibited weak green fluorescent light, indicating early apoptosis; and when the concentration of 3-MCPD raised to 40 mM, the treated cells exhibited intense red fluorescent light, implying apoptosis death. The apoptosis rate generated by the HCS analysis software is shown in Fig. 1D and the apoptosis rate of cells treated with 40 mM 3-MCPD for 24 hr reached approximately 35%.

3-MCPD induces oxidation stress, DNA damage and abnormal cell cycle

Using DCFH-DA, we observed ROS level in HEK293 cells to evaluate its direct effect on 3-MCPD-induced oxidation stress. We harvested cells to evaluate ROS level after incubation with 3-MCPD (5, 10 and 20 mM) for 24 hr. A marked increase in ROS level appeared following 20 mM 3-MCPD treatment, approximately three-fold that of unexposed control cells (Fig. 2A and B). This phenomenon indicates ROS formation is involved in 3-MCPD-induced apoptosis in HEK293 cells.

By applying the neutral version of the comet assay to determine strand breaks and a few apurinic/apyrimidin-

ic (AP) sites (Collins, 2004), we measured DNA damage in HEK293 cells treated with 3-MCPD (Fig. 2C). In the comet assay, damaged DNA fragments migrate out of the cell nucleus as a streak similar to the tail of a comet, and the quantified olive tail moment (OTM) is directly proportional to the amount of DNA damage (Kent *et al.*, 1995). The mean OTM value of the negative control is 3.53 ± 0.53 (SD). Rising 3-MCPD concentration signals an increase in DNA damage. Moreover, cells treated with 10 mM and 20 mM 3-MCPD show greater OTM value. Our results indicate that 3-MCPD induces oxidative DNA damage in HEK293 cells. As it is reported, ROS can trigger the release of mitochondrial cytochrome C, contributing to apoptosis (Kulms *et al.*, 2002).

The cell cycle of HEK293 caused by different concentrations of 3-MCPD was evaluated by flow cytometry, as shown in Fig. 2D. Induction of apoptosis and/or inhibition of cell proliferation are highly correlated with the activation of a variety of intracellular signaling pathways leading to arrest the cell cycle in the G_1 , S, or G_2/M phase of the cell cycle. After treatment with various concentrations of 3-MCPD (0, 5, 10, 20 mM) for 24 hr, Cell Quest software was applied for analysis of the cell cycle. With the

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Fig. 2. Using DCFH-DA, observation of ROS level in HEK293 cells to evaluate its direct effect on 3-MCPD-induced oxidation stress (A) and (B), data are expressed as the mean ± S.D. (C) Representative comet images of HEK293 cells in control and incubation with 3-MCPD for 24 hr, and extent of DNA damage of 3-MCPD on HEK293 cells in the form of table. (D) Changes of cell cycle of HEK293 caused by different concentrations of 3-MCPD. Dunnett post test was applied for comparison all dosed-groups with control group, * for P < 0.05, and ** for P < 0.01.

increase of 3-MCPD, percentage of G₀/G₁ phase down-regulated, but percentage of S phase and G₂/M phase up-regulated, progressively. The cell number of G₂/M phase increased, treated with 20 mM 3-MCPD for 24 hr comparing with the control group, which illustrated that cell was block in G₂/M phase and proliferation significantly decreased. Presumably, apoptosis induced by 3-MCPD is a consequence of G₂/M block; certainly, the DNA dam-

age could also due to abnormal mitotic arrest (Jordan *et al.*, 1996).

Effect of 3-MCPD on Caspase-3, Caspase-8 and Caspase-9 activation

Caspases orchestrate the dismantling and clearance of apoptotic cell (Ricci *et al.*, 2004). Among them, caspase-3 appears to be responsible for the apoptotic hall-

marks. An increase in caspase-3 activity was observed in 3-MCPD treated cells as compared to controls. The relative caspase-3 enzyme activity of cells treated 20 mM 3-MCPD for 24 hr doubled approximately than that of control group, which illustrated that the cell apoptosis process was stimulated by 3-MCPD. The caspase-9 involved in the mitochondrial apoptotic pathway (Krajewski *et al.*, 1999). When the mitochondrial pathway of cell apoptosis was triggered, the cytochrome C (CYC) and apoptosis-related factors will be released from mitochondria as a kind of apoptotic bodies, which are activated conformational changes (Du *et al.*, 2000). As observed from Fig. 3A, the caspase-9 activity was increased treated the 3-MCPD. Caspase-8, FADD death domain homologous protein enzyme, would be activated by apoptosis receptor, like Fas, FasL, TNFR, *et al.* Activated caspase-8 broken Bid to tBid in the cytoplasm, and the tBid, transferred into the mitochondria, could induce the release of CYC. Caspase-8 and caspase-9 enzyme activity were stimulated simultaneously by 3-MCPD, which indicated that 3-MCPD could induce the HEK293 cell apopto-

sis through the mitochondrial pathway and death receptor pathway.

Effect of 3-MCPD on Bcl-2, Bax, P53 and CYC activation

The anti-apoptotic Bcl-2 protein, the role of mitochondria to maintain the normal function of mitochondria, and Bax, a pro-apoptotic protein, were evaluated through western blot, as shown in Fig. 3B. Bax/Bcl-2 ratio was regarded as a value to monitor the cells apoptosis (Chresta *et al.*, 1996). The increase of Bax expression and the decrease of Bcl-2 of Bcl expression by 3-MCPD indicated the activation of the mitochondrial apoptosis pathway. After p53 activation by 3-MCPD, the activation of pro-apoptotic proteins downstream would induce apoptosis; therefore, it could be considered that upstream of p53 gene in apoptotic cascade could activate downstream apoptosis execution protein induce the cell apoptosis. When the cell apoptosis process started, the CYC protein was released into the cytoplasm from mitochondria as a kind of apoptosis inhibitory protein. In Fig. 3B, the up-

Fig. 3. (A) Effects of different concentrations of 3-MCPD (5 mM, 10 mM and 20 mM) for 24 hr on caspase-3/8/9 activity levels, and (B) Effect of different concentrations of 3-MCPD (5 mM, 10 mM and 20 mM) for 24 hr on expression of apoptosis-related proteins, including Bcl-2, Bax, P53 and CYC. Data are expressed as the mean $\pm$ S.D., Dunnett post test was applied for comparison all dosed-groups with control group, * for $P < 0.05$, and ** for $P < 0.01$.

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regulation of CYC protein induced by 3-MCPD foreboded the activation of cell apoptosis pathway. Then changes of apoptosis-related genes of cell treated by 3-MCPD were analyzed by microarray.

Effect of 3-MCPD on apoptosis pathway of HEK293 cells

To further analyze 3-MCPD-induced apoptosis-related gene mechanism, a total of 84 genes associated with apoptosis, including BCL2 family, Caspase apoptosis-related cysteine family, TNF tumor necrosis family, TNF receptor family, CARD death domain family, CYC, *et al.* were detected through an apoptosis signaling pathway mRNA expression microarray. Compared with the control group, 9 apoptotic genes were up-regulated more than 2-fold and 6 down-regulated more than 2-fold (Table 1). Genes associated with the mitochondrial apoptotic pathway, BCL2 family, including BID, changed significantly, indicating that the mitochondrial apoptotic pathway is activated. Death receptor pathway-related genes, TNFRSF11B and TNFRSF1A, changed significantly, indicating that the death receptor pathway is also activated, as shown in Fig. 4A. The effect of 3-MCPD on HEK293 cell apoptosis-related gene mRNA levels is shown in Fig. 4B. The location information genes' names are shown in Table S2 in supplementary file.

Current research has been confirmed that there are three mainly apoptotic signaling pathways: mitochondrial pathway, death receptor pathway and endoplasmic reticulum pathway. The three pathways involved different genes, but the final execution of apoptosis proteins were caspase enzymes family. Death receptor pathway

was closely related to receptor activation (Ashkenazi and Dixit, 1998). As seen in Fig. 5, when cell was stimulated with 3-MCPD, some death receptors, FAS, TNFR1, and their ligand (FASL, TNF- α) were activated, which would raise Fas-associated death domain protein FADD to connect with pro-caspase-8. It occurs self-cut and activation due to local concentration increased, which can lead to the activation of Caspase-8, triggering a cascade reaction of caspase, like pro-caspase-3 and caspase-3. FADD and caspase-8, especially, played an important role in many apoptotic pathways modulated by death receptors. Furthermore, the active caspase-8 could cut N-terminal amino acid cytoplasmic proteins, making Bid cleavage tBid protein, which was then transported from the cytoplasm to the mitochondria. BCL-2 family proteins interacted to form the mitochondrial membrane channel, causing the release of CYC from mitochondria. Released CYC participated with ATP, can reacted with apoptosis-related protein factor (Apaf-1) as oligomerization to form Apaf-Cytc complexes. The incessancy aggregation of Pro-caspase-9 can lead to local self-cleavage and activation, activating caspase-9 and its downstream protein, causing caspase cascade action and triggering apoptosis. Microarray report found a significant change in a gene, named growth arrest and DNA damage 45a gene (GADD45a), which had relationship with cell cycle (Gao *et al.*, 2005). GADD45 family were the downstream genes of P53 gene, which could be induced by DNA damage (Kastan *et al.*, 1991). GADD45a expression level increased with cell block in G₂/M phase, manifesting as impeding cell growth. This phenomenon was according to the results of DNA damage and abnormal cell cycle above. To sum up, the exper-

Table 1. The name and numerical changes of genes reported on microarray.

Gene symbol	Gene related protein / Definition	Fold change Dose / Control	P value
PYCARD	Apoptosis-associated speck-like protein containing a CARD	9.41	0.02
LTA	TNF Ligand Family	7.85	< 0.01
CD27	TNF Ligand Family	4.29	< 0.01
CYCS	Cytochrome c, somatic	2.53	< 0.01
BIK	BCL2-interacting killer (apoptosis-inducing)	2.07	< 0.01
MCL1	Myeloid cell leukemia sequence 1	4.2	< 0.01
GADD45a	Growth arrest and DNA-damage-inducible 45 alpha	10.09	< 0.01
TNFRSF11B	Tumor necrosis factor receptor superfamily, member 11b	2.56	< 0.01
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A	2.75	< 0.01
TNFSF8	tumor necrosis factor superfamily member 8	0.18	0.02
AIFM1	apoptosis inducing factor, mitochondria associated 1	0.49	0.04
CD70	Cytokine-cytokine receptor interaction	0.4	< 0.01
CRADD	CASP2 and RIPK1 domain containing adaptor with death domain	0.31	< 0.01
IGF1R	Insulin-like growth factor 1 receptor	0.38	< 0.01
BIRC3	Apoptosis, TNF signaling pathway	0.42	< 0.01

Fig. 4. (A) 3D map results due to chip and location information and (B) effect of 3-MCPD on HEK293 cell apoptosis-related gene mRNA levels.

Fig. 5. The 3-MCPD-induced apoptosis pathway of HEK293 cells.

iment results indicated that 3-MCPD induced HEK293 cell toxicity through the death receptor pathway and mitochondrial pathway.

In conclusion, in order to further understand mechanism of 3-MCPD-induced apoptosis on HEK293 cell, cell viability, DNA damage, intracellular ROS activity and apoptosis-related protein was evaluated. Genes related with apoptosis were detected by qPCR-array for speculation of the 3-MCPD-induced cell apoptosis signaling pathway. Our results clearly showed that 3-MCPD treat-

ment inhibits cell proliferation and reactive oxygen species generation. Genes associated with the mitochondrial apoptotic pathway, BCL2 family and death receptor pathway-related genes, changed significantly, indicated that the mitochondrial apoptotic pathway and death receptor pathway were activated, respectively, resulting in the inhibition of cell growth and proliferation as well as induction of apoptosis. The experiment results indicating that 3-MCPD induced HEK293 cell toxicity through the death receptor pathway and mitochondrial pathway.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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