Perfluorooctanoic acid stimulates breast cancer cells invasion and up-regulates matrix metalloproteinase-2/9 expression mediated by activating NF-κB

Weidong Zhang, Pengliang Wang, Pengfei Xu, Chen Miao, Xin Zeng, Xianwei Cui, Cheng Lu, Hui Xie, Hong Yin, Fei Chen, Jingjing Ma, Sheng Gao, Ziyi Fu

* Nanjing Maternal and Child Health Medical Institute, Affiliated Nanjing Maternal and Child Health Hospital, Nanjing Medical University, Nanjing 210004, China
** Department of Ophthalmology, The First Affiliated Hospital, Nanjing Medical University, Nanjing 210029, China
*** Department of Breast Surgery, Affiliated Nanjing Maternal and Child Health Hospital, Nanjing Medical University, Nanjing 210004, China
**** Department of Cell Biology, School of Basic Medical Sciences, Nanjing Medical University, Nanjing 210029, China

HIGHLIGHTS

- PFOA exposure (≥5 nM) evidently enhanced the invasion ability of the breast cancer cells MDA-MB-231.
- MMP-2/9 of MDA-MB-231 cells were increased with PFOA treatment.
- PFOA could activate nuclear factor kappaB (NF-κB) by accelerating NF-κB translocation into the nucleus.
- NF-κB inhibitor could reverse PFOA-induced breast cancer cells invasiveness enhancement and MMP-2/9 overexpression.
- PFOA can stimulate breast cancer cells invasion and up-regulates matrix metalloproteinase-2/9 expression mediated by activating NF-κB, which deserves more environmental health concerns.

ARTICLE INFO

Article history:
Received 6 November 2013
Received in revised form 25 May 2014
Accepted 1 June 2014
Available online 21 June 2014

Keywords:
Perfluorooctanoic acid
Breast cancer
Invasion

ABSTRACT

Perfluorooctanoic acid (PFOA) is widely used because of its stain-resistant and water-repellent properties. This study aimed to explore the molecular mechanisms underlying the stimulation effects of PFOA on cancer cell invasion and matrix metalloproteinases (MMPs) expression. Trans-well filter assay showed that PFOA exposure (≥5 nM) evidently enhanced the invasion ability of the breast cancer cells MDA-MB-231. Luciferase reporter assay, quantitative real-time PCR, western blotting and gelatin zymography consistently demonstrated that mRNA and protein levels of MMP-2/9 were increased in the cells after PFOA treatment (P < 0.05 each). Western blotting revealed that PFOA could activate nuclear factor kappaB (NF-κB) by accelerating NF-κB translocation into the nucleus. Furthermore, addition of NF-κB inhibitor in culture medium could suppress the breast cancer cells invasiveness enhancement and MMP-2/9 overexpression. This study indicates that PFOA can stimulate breast cancer cells invasion and up-regulate matrix metalloproteinase-2/9 expression mediated by activating NF-κB, which deserves more environmental health concerns.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Perfluorooctanoic acid (PFOA), a man-made chemical, is frequently used in industrial and consumer products, corresponding to its stain-resistant and water-repellent characteristics. So far, it has been detected in everything surrounding us, such as non-
stick cookware, cosmetics, upholstery, bio-accumulate in food chains (Bartell et al., 2010; Fraser et al., 2012), even in drinking water (Ji et al., 2011). As known, the concentration of PFOA existed in drinking water varied from picogram per liter to microgram per liter (Koehler and Jensen, 2009), meanwhile, regression analysis indicated that PFOA contamination increasing on an average of 7–11% per year during past decades (Koehler and Jensen, 2009).

More importantly, several reports demonstrated that PFOA contamination of human serum attained on an average of 2.82 ng/ml (approximately 68.1 nm) (Ji et al., 2010). Because of its widespread occurrence, it has attracted much concentration since 2001. Recent epidemiological studies have linked the toxicity of PFOA to tumorigenesis, such as pancreatic cancer, liver cancer, testicular cancer (Saucier et al., 2007; Viets et al., 2017), and probably breast cancer (Büttner-Jongen et al., 2011). However, information about the relationship between PFOA exposure levels and tumor metastasis is omitted, although tumor metastasis is an important section during carcinoma progression, which might cause the majority of cancer treatment failure and poor prognosis (Chambers et al., 2002; Sorg et al., 2015).

Cancer metastasis undergoes a series of steps including vessel formation, cell attachment, invasion and cell proliferation (Sobolov and Popov, 2008). Cancer cells must migrate through and digest surrounding tissue barriers to escape from the primary site and form the distant organ colonization, so the degradation of basement membranes and extracellular matrix (ECM) is a crucial step for tumor metastasis (Sobolov and Popov, 2008). This procedure requires different cellular proteolytic enzymes, including matrix metaproteases (MMPs) family, which is one of the important families of proteases responsible for the ECM destruction and rebuilding. The MMPs are a family of 24 human zinc-binding endopeptidases which can degrade virtually all extracellular matrix (ECM) components (Sobolov and Popov, 2008). These MMPs are synthesized in most cells and immediately secreted into the ECM (Sobolov and Popov, 2008). They play an important role in various cellular processes including embryogenesis, inflammation, wound healing, arthritis, cardiovascular diseases and cancer invasion (Sobolov and Popov, 2008). Among these MMPs, MMP-2 (72 kDa gelatinase) and MMP-9 (92 kDa gelatinase), which can efficiently degrade native collagen types I, IV, fibronectin, entactin, and elastin, are well known to play critical roles in cancer progression, such as invasion and distant metastasis of breast cancer as well (Friedman and Viedt, 2008). Meanwhile, MMP-2/9 overexpression is closely related to poor prognosis in patients.

MMP-2/9 expression could be regulated by nuclear factor kappaB (NF-κB) (Chen et al., 2010). NF-κB is an important transcription factor that controls several cellular responses including invasion, inflammation (Furneaux and Groves, 2009), apoptosis, and survival (Furneaux and Groves, 2009). Once the activated NF-κB complex translocates from cytoplasm to nucleus and bind to target genes, it could initiate the gene transcription (Furneaux and Groves, 2009). Corsini et al. found that PFOA could regulate LPS-induced MMP-9 release (Corsini et al., 2010). These studies have indicated that NF-κB activation contributes to the enhanced invasiveness of carcinoma cells by regulating MMP-9 and MMP-2 expression (Chen et al., 2010; Chen et al., 2008), and previous studies have indicated that NF-κB activation contributes to the enhanced invasiveness of carcinoma cells by regulating MMP-9 and MMP-2 expression (Chen et al., 2008; Chen et al., 2010), while the effects of environmental pollutants on cancer cell invasion via this pathway are still unknown. In this study, we have demonstrated that low concentration of PFOA contributed to enhancing MDA-MB-231 breast cancer cells invasiveness, so we further explored the role of NF-κB in the underlying molecular mechanism mediating the overexpression of MMP-2/9 and enhancement of invasiveness of the breast cancer cells exposed to PFOA.

2. Materials and methods

2.1. Reagents and antibodies

The PFOA purity ≥ 95% by HPLC was purchased from Sigma (USA). MDA-MB-231 human breast cancer cell line was obtained from the American Type Culture Collection. Cell culture medium DMEM was purchased from Gibco (Invitrogen China Limited, China). All antibodies used in this study were provided by Cell Signaling Technology (USA). Other reagents were provided by Sigma-Aldrich Inc. (USA) unless otherwise mentioned.

2.2. Cell culture and PFOA exposure

Human breast cancer cells MDA-MB-231 were maintained in DMEM medium containing 100 U/ml streptomycin and 100 U/ml penicillin supplemented with 10% fetal bovine serum (FBS). Cells were cultured in a humidified atmosphere of 5% carbon dioxide (CO2) at 37°C. PFOA was dissolved in sterile water to make a 2 mM stock solution and added to the media to reach different doses (0, 0.1, 1, 3, 10, and 50 μM). In order to explore the role of NF-κB in the molecular pathway mediating the promoted cell invasion and MMP-2/9 expression, JSH-23 was dissolved in dimethyl sulfoxide (DMSO) and added to the culture media with final concentration of 10 μM (equal DMSO added to culture medium as solvent control groups).

2.3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT experiment was performed to judge the effect of PFOA on cell proliferation viability (Chen et al., 2010). Human breast cancer cells MDA-MB-231 were seeded in a 96-well plate with serum-free medium, 5000 cells/well, and 24 h later the cells were treated with different doses of PFOA. At certain time points (72 h), the cells were incubated in 0.83 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in growth medium at 37°C for 4 h and lysed in 100 μL of dimethyl sulfoxide at room temperature for 15 min. The absorbance in each well was measured at 490 nm by Synergy 2 Absorbance Microplate Reader (Biotek, USA). Cell growth inhibition rate was expressed as a fraction of absorbance compared to control.

2.4. Cell invasion ability assay

After PFOA exposure, trans-well filter assays were conducted to investigate MDA-MB-231 invasion ability by using 24-well ECM-coated trans-well inserts (Chen et al., 2010). Briefly, the upper surface of the filter (8.0 μm in pore size) was coated with 80 μg ECM gel before being air-dried overnight at 37°C. Approximately 4 × 104 cells were added to the upper chamber, cultured in serum-free DMEM culture medium and DMEM with 10% FBS was added to the lower chamber. For drug inhibition experiments, JSH-23 (10 μM) was added to the upper filters prior to invasion assays, and equal DMSO was added to culture medium of another group as solvent control. After 72 h incubation with different doses of PFOA in an environment of 5% CO2 at 37°C, cells degraded the ECM and adhered to the opposite surface of the filter. The filters were then fixed with 4% paraformaldehyde (dissolved in PBS) and stained with crystal violet before the cells on the upper surface were removed completely with a cotton swab. The cells, which translocated from the upper to the lower side of the filter, were observed under light microscopy at a magnification of ×100. Cells were then lysed by cell lysis buffer and collected for the detection of optical density (OD) at 570 nm. OD values of the cells treated with different concentrations of PFOA were normalized to those of
the control cells (no PFOA treatment). Breast cancer cells MDA-MB-231 invasive ability was defined as the mean OD volume of cells.

2.5. RNA extraction, reverse transcription PCR, and quantitative real-time PCR

We employed quantitative real-time PCR (qRT-PCR) to detect the mRNA expression levels of homo-mmp-2/9, with homo-β-actin as housekeeping genes. After the cells were treated with PFOA for 72 h, total RNA was isolated from breast cancer cells with Trizol reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). The purified total RNA (1 μg) was then reversely transcribed using the First Strand cDNA Synthesis Kit (TaKaRa, Japan). The qRT-PCR primers of mmp-2 gene were: forward 5'-CAG AAC ACT CTC AAG AG-3' and reverse 5'-GGA CTC AAG ATT AC-3'. The primers of mmp-9 gene were: forward 5'-TCC GGC CAC GTA GAC-3' and reverse 5'-TCA GGG CCA GGA CCA TAC AG-3'. PCR primers set of the internal control gene β-actin were: forward 5'-TCA GGC CAC GTA GAC-3' and reverse 5'-CTG GAA GGT GAA CCA GG-3'. Reactions were conducted in 96-well plates with a total volume of 20 μL including 10 μL SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA), plus 1 μL each primer (2 μM), 1 μL template DNA, and 7 μL ddH₂O. Thermal cycling and fluorescence detection were conducted on an Applied Biosystems Viia™(Life Technologies, USA), using the following protocol: 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. Each reaction was run in triplicate. The levels of mmp-2/9 gene expression were normalized to β-actin levels using the method of 2 ^ΔΔCt^ as assessed by electrophoresis of PCR products, no primer–dimer was observed for both the target genes and β-actin, and the specificity of the products was also confirmed by melt curve analysis.

2.6. Luciferase assay

Luciferase reporter assay experiment was conducted to perform the effect of PFOA on the gene promoter activity of mmp-2/9. MDA-MB-231 cells were grown on a 24-well plate for 24 h to reach approximately 70% confluence prior to transfection. The cells in each well were co-transfected with 0.4 μg of mmp-2/9 promoter site-derived luciferase reporter plasmid and 0.1 μg of β-galactosidase (β-gal) expression plasmid using Lipofectamine 2000 (Invitrogen, China Limited, China) according to the manufacturer's protocol. After transfection for 6 h, cells were treated with different doses of PFOA, JSH-23 (10 μM) or both JSH-23 (10 μM) and PFOA (50 nM) (equal DMSO was added to culture medium of another group as solvent control). 24 h later, cell lysates were prepared with Reporter Lysis Buffer (Promega, Madison, WI) following the manufacturer's instructions, and luciferase activity was measured with Luciferase Assay Reagent 10-Pack (Promega). The β-gal activity of the cells was measured as follows: 1 μL of Mg solution (0.1 M MgCl₂, 4 M beta-mercaptoethanol), 22 μL of 4 mg/mL O-nitrophenol-β-galactosidase solution (O-nitrophenol-beta-galactopyranoside) (in 0.1 M phosphate buffer (pH 7.5)), and 57 μL of 0.1 M phosphate buffer (pH 7.5), were added into the obtained cell extract solution (20 μL) to reach a total volume of 100 μL. As control, the optical density (OD) value of β-gal at 450 nm was measured in parallel.
2.7. Western blotting

After the cells' protein components were collected, a standard western blotting analysis was used to investigate the MMP-2/9 and NF-κB protein expression levels. Briefly, nuclear and cellular protein extracts were prepared, 72 h after treatment with different doses of PFOA, JS2-23 (10 μM) or both JS2-23 (10 μM) and PFOA (50 nM) (equal DMSO was added to culture medium of another group as solvent control), by using KEYGEN (Protein Extraction Kit (KEYGEN Nanjing). For detecting the ratio of nuclei (p65) cytosol (p65), cytosol protein and nucleus protein were dissolved in equal volume lysis buffer of each group cells. Protein lysates were boiled in SDS-sample buffer for 5 min and then subjected to 8% SDS-polyacrylamide gel electrophoresis and transferred to PVDF (polyvinylidene fluoride) membranes (Bio-Rad). Membranes were then blocked for 2 h in 5% milk-Tris-Buffered Saline Tween-20 (TBST) at room temperature, and incubated overnight at 4°C either with the monoclonal antibodies (Cell Signaling Technology, USA) of anti-β-actin, anti-MMP-2, anti-MMP-9 or anti-NF-κB. Membranes were washed four times in TBST and incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 h. Blots were visualized by enhanced chemiluminescence (Thermo, USA) and analyzed using a scanning densitometer with the molecular analysis software FluorChem M system (Protein Simple, USA).

2.8. Gelatin zymography

The enzyme activities of MMP-2 and MMP-9 were analyzed by gelatin zymography. Cells (4 x 10^5) were seeded into a 24-well plate and allowed to adhere for 6-8 h with the presence of serum. Subsequently, the culture medium was replaced by serum-free DMEM medium (400 μL per well) with different doses of PFOA (0, 0.1, 1, 5, 10, and 50 nM). After incubation for 72 h, the medium in each well was gathered and centrifuged for 10 min at 2000 rpm to remove cell debris. The supernatant was collected and mixed with sample loading buffer containing 0.1% SDS-polyacrylamide gel (containing 1 mg/mL gelatin) electrophoresis. Then the gel was soaked in 2.5% Triton-X-100 for 30 min twice to remove SDS, and transferred to bath buffer containing 50 mM Tris (pH 8.0), 5 mM CaCl2 and 2 mM ZnCl2 at 37°C for 16 h. The gel was then stained with 0.1% Coomassie blue in 45% methanol and 10% acetic acid. The visualized bands indicating the presence of a protein with gelatinolytic activity were analyzed by the FluorChem M system (Protein Simple, USA).

2.9. Statistical analysis

The differences between the treated and the control cells the invasion rates and protein expressions were by applying one way ANOVA (analysis of variance) method followed by post-hoc Tukey's
analysis. A p-value of less than 0.05 was considered statistically significant, each experiment was performed at least triple times. Computer-based calculations were conducted using SPSS version 20.0 (SPSS Inc. Chicago, Illinois, USA).

3. Results

3.1. Effects of PFOA treatment on breast cancer cell invasion

In order to investigate the invasiveness of the breast cancer cells (MDA-MB-231) after different doses of PFOA treatment, we performed matrigel invasion assays. First, we evaluated the effect of different doses of PFOA on cell survival rates. High-dose PFOA treatments (>100 nM) could inhibit the viability of MDA-MB-231 cells (P < 0.05), but no cell viability inhibition was observed after treatment with low concentrations (<100 nM) (P > 0.05) (Fig. 1A). Furthermore, we found that low-doses exposure could stimulate the invasion ability of the crystal violet stained cancer cells (Fig. 4B), which was subsequently confirmed by determining the OD value of the invaded cells (Fig. 3C). PFOA treatments at 5, 10, or 50 nM significantly increased the number of the invaded cells (P < 0.05), while treatment at 0.1 or 1 nM caused no change on cell invasion ability (P > 0.05). Compared with the control, cell invasion was significantly enhanced by 1.5, 2.17 and 3.14 fold after PFOA exposure at 5, 10 and 50 nM. PFOA stimulated the cancer cells invasion in a dose-dependent manner (R² = 0.8422).

3.2. Enhanced MMP-2/9 expression and activation levels in PFOA-treated MDA-MB-231 cells

To confirm the effect of the PFOA treatment on MMP-2/9 expression, we investigated the gene promoter activity, mRNA and protein expression levels of MMP-2/9 in MDA-MB-231 breast cancer cells after 24 h treatment with different doses of PFOA. qRT-PCR results demonstrated that the PFOA treatment upregulated gene mmp-2/9 expressions (P < 0.05). Low-dose of PFOA (< 5 nM) showed no effect on mmp-2/9 mRNA levels, however, higher-dose of PFOA (5–50 nM) increased MMP-2/9 mRNA expressions (P < 0.05). Western blotting showed that the protein levels of MMP-2/9 were also increased after PFOA treatments at 5 nM (Fig. 4B), which was further supported by luciferase reporter assay (Fig. 3C). Meanwhile, gelatin zymography analysis data demonstrated that MMP-2/9 activation levels were increased after PFOA treatment (P < 0.05). Results of qRT-PCR, western blotting, luciferase reporter, and gelatin zymography results revealed that PFOA exposure could evidently up-regulate both expression and activation of MMP-2/9 in MDA-MB-231 cells, which may be subsequently involved in the cell invasion promotion.

3.3. NF-κB activation in PFOA-treated cells

We further examined the effect of PFOA treatment on NF-κB activity to explore the potential molecular mechanisms of MMP-2/9 overexpression. The cytosol and nuclear proteins were isolated from the PFOA-treated cells for western blotting of p65 (Fig. 4A). The data showed that after PFOA treatment, ratio of the nucleus/cytosol level of NF-κB was significantly elevated from 0.24 ± 0.04 (the control) to 0.69 ± 0.12 (50 nM PFOA) (Fig. B) (P < 0.05).

3.4. Mediation of NF-κB in MMP-2/9 expression and cell invasion

Drug inhibition experiment was performed to confirm the role of NF-κB activation in the MMP-2/9 stimulation and cell invasive ability promotion. First, we confirmed that after NF-κB inhibitor JSH-23 (10 μM) treatment, nuclear/cytosol ratio of NF-κB was significantly decreased 12% compared to control group; we also found that after JSH-23 pre-incubation, nucleus/cytosol ratio of NF-κB was only up-regulated 16% with PFOA exposure, while PFOA increased 36% NF-κB nuclear translaction without JSH-23 pre-incubation, which indicated that NF-κB inhibitor JSH-23 partially inhibited PFOA-induced NF-κB nuclear translaction (Fig. A). Then we performed trans-well filter assay to certify the inhibition effect of JSH-23 on PFOA-induced MDA-MB-231 invasion. The data showed that with JSH-23 pre-treatment, PFOA-promoted cancer cell invasiveness was reduced significantly (Fig. 4B), which suggested that PFOA could activate cancer cell invasion through inducing NF-κB activation. Meanwhile, JSH-23 could reverse the stimulation effect of PFOA on gene MMP-2/9 promoter activities (Fig. 3C) and expression levels (Fig. D) by using luciferase reporter assay and qRT-PCR, which was consistent with western blotting data (Fig. 4E). Furthermore, gelatin zymography demonstrated that MMP-2/9 activity was inhibited in the cells treated with both JSH-23 and PFOA (Fig. 4F), suggesting that NF-κB mediated MMP-2/9 overexpression and enzyme activation stimulated by PFOA.

4. Discussion

This study demonstrated that the environmental pollutant PFOA can increase invasiveness of breast cancer cells. Accumulating evidence has shown that PFOA can act as a tumor initiator to induce DNA damages and oxidative stress (Weiss et al., 2010; Yao and Zhang, 2008). However, little information is available about the effect of PFOA on breast cancer cell invasion, although clinical concerns have focused on screening and developing drugs capable of inhibiting breast cancer metastasis (Wang et al., 2010; Yeh et al., 2010). Recent studies have indicated that cancer cell migration and invasion progression can be induced by physicochemical factors.
such as progesterone receptor (Yu et al., 2010), glutamate (Hernor et al., 2011), extracellular calcium (Salatik et al., 2008), bacterial peptidoglycan (Xie et al., 2010), microcystin-LR (Zhang et al., 2012) and so on, but there is little information reported on the relationship between environmental pollution and breast cancer metastasis.

Our results suggested that low-dose treatments (<100 nM) could not affect breast cancer cell MDA-MB-231 survival, but high-dose treatments (>100 nM) could suppress cell viability. In order to exclude the influence on cell survival, we chose the low-dose groups (0–50 nM) to explore the effect and mechanism of PFOA on breast cancer cells invasion ability. In the present study, invasive ability of breast cancer cell MDA-MB-231 was significantly enhanced after PFOA exposure. MMP-2/9, which can efficiently degrade extracellular matrix, are well known to play critical roles in breast cancer invasiveness. In order to elucidate the underlying molecular mechanisms, we detected the influence of PFOA treatment on the promoter activity, mRNA expression, protein expression and enzyme activation levels of MMP-2/9 by using luciferase reporter assay, qRT-PCR, western blotting and gelatin zymography separately. Results consistently showed that PFOA could enhance MMP-2/9 expression and enzyme activation levels in the breast cancer cells.

MMPs over-expression is closely related to tumor metastasis, much effort has been devoted to screening and developing drugs which can inhibit MMPs expression (Lee et al., 2008). However, few environmental pollutants have been known to be capable of inducing MMPs expression. Recently, microcystin-LR has been well documented to induce MMP-2/9 expression through activation of NF-κB in human melanoma cells (Stram et al., 2010). Apart from chemicals, radiation can also up-regulate MMP-9 mRNA level, protein level and catalytic activity by activating NF-κB in hepatocellular carcinoma cells (Corsi et al., 2007). Corsi et al. have found that 100 μg/ml (approximately 0.24 mM) PFOA could reduce LPS-induced MMP-9 via regulating NF-κB phosphorylation level, while in this study we found that environmental concentration (nM level) of PFOA could induce NF-κB activation, which might be involved in the over-expressions of MMP-2/9. NF-κB complex located in the cytoplasm in a latent form often transfers to the nuclear when NF-κB was activated, so we detected the changes of NF-κB activity by assessing the ratio of nuclear (NF-κB):cytosol (NF-κB). Western blotting showed that PFOA treatment induced nuclear translocation of NF-κB, revealing that NF-κB activation might mediate MMP-2/9 overexpression induced by PFOA.

The proposed molecular mechanism was further confirmed by the JSH-23 suppression assay, a well-established NF-κB inhibition
method for MDA-MB-231 cells. The increased MMP-2/9 activity and invasiveness were inhibited by JSH-23 in the breast cancer cells cultured with PFOA, suggesting that NF-kB plays a crucial role in the MMP-2/9 overexpression and invasiveness enhancement.

NF-kB is an important transcription factor controlling cell apoptosis and survival (1). This study showed that the increase of NF-kB activity could promote MMPs expression and breast cancer cell invasion, but did not affect cell viability, which is supported by the study of Zhang et al. (2).


It has been identified that thioridoxin can stimulate MMP-9 transcription through activating NF-kB in breast cancer MDA-MB-231 cells (3). NF-kB also could mediate MMP-2 expression and activation through stimulating MT1-MMP expression (4) and activating pro-MMP-2 (5). Taken together, PFOA can activate NF-kB to induce MMP-2/9 overexpression, resulting in enhanced breast cancer cell invasion. PFOA is widely used because of its stain-resistant and water-repellent properties, and given its persistence, it is ubiquitous in many indoor environments, including homes and workplaces (6). The NF-kB pathway and its related components are involved in various inflammatory and immune responses, and our findings point to the importance of identifying the induced public health risks in our surrounding environments.

In conclusion, this study suggests that PFOA exposure can promote breast cancer cell invasion by stimulating MMP-2/9 mRNA expression and activation. PFOA is able to activate NF-kB by stimulating translocation into the nucleus. The NF-kB may contribute to the MMP-2/9 overexpression and cancer cell invasiveness enhancement.

Conflict of interest

The authors declare that they have no competing interests.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgments

This study was financially supported by the Key project of the National Natural Science Foundation of China (81330067), the National Natural Science Foundation of China (81302304, 81302306, and 81172501), the Natural Science Foundation of Jiangsu Province (BK20130074).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.toxlet.2014.06.004.

References

[References are not visible in the provided image.]
Bibliography or references are not visible in the provided image.